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ASSESSMENT OF PROGNOSTIC FACTORS IN PLASMA CELL MYELOMAS ON TISSUE MICROARRAYS USING IHC AND FISH ANALYSIS

Bettina Jenni[1,2], Tanja Reineke[1], Dimitri Korol[2], Valentin Rousson[3], Corina Dommann-Scherrer[4], Robert Maurer[5], Holger Moch[1], Nicole M. Probst-Hensch[2] and Marianne Tinguely[1]

Institute of Surgical Pathology[1], University Hospital Zurich; Department of Molecular Epidemiology/Cancer registry[2], University of Zurich; Institute of Social and Preventive Medicine[3], University Zurich; Institute of Pathology Winterthur[4]; Institute of Pathology Triemli[5]

Abstract

Plasma cell myelomas (PM) exhibit a clinical and molecular heterogeneity. So far, traditional morphology and immunohistochemistry (IHC) were only of limited value to stratify patients into different prognostic or predictive categories. Among hematological neoplasms, PMs are unique for their extensive genomic instability, involving both numerical and structural rearrangements. Chromosomal translocation involving the IgH locus at 14q32 are seen in most cases of PM. In the last years, some of these translocations have gained prognostic and/or a predictive importance. Interphase Fluorescence in Situ Hybridization (FISH) analysis allows to specifically search for distinct translocations in formalin fixed, paraffin embedded tissues. We therefore compared FISH analysis with classical morphology and IHC for prognostic significance on a tissue microarray (TMA) in 135 archival specimens from 119 patients with PM.

Material and Methods

Study population and tissue samples

135 archival biopsies of 119 patients with PM diagnosed between 1983 and 2003 were retrieved from the files of the Institutes of Surgical Pathology, University Hospital Zurich, town hospital Triemli and canton hospital Winterthur (Canton of Zurich, Switzerland). Of the 135 surgical biopsies, 127 (94%) were of osseous and 8 (6%) of extra-ousseous origin. Survival data was available for 111 patients. Their follow up time ranged from 1 week to 14.33 years.

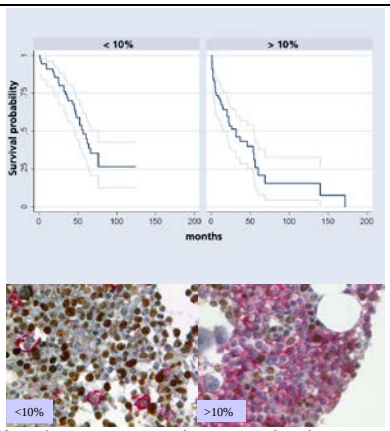
Immunhistochemistry

Immunohistochemistry (IHC) on TMA sections was performed by an automated immunostainer (Ventana Medical System, Tucson, AZ), including the antibodies anti-CD20, CD79a, CD38, CD138, CyclinD1and Mib1.

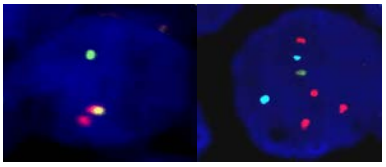
Double stainings for CD138/Mib and CD138/CyclinD1 were carried out.

Interphase FISH and FICTION

Interphase FISH was combined with immunofluorescence staining for cytoplasmic light chain immunoglobulins (FICTION) to identify the chromosomal abnormalities exclusively in the malignant plasma cell clone. For the detection of these chromosomal abnormalities, the commercially available dual color, dual fusion translocation probes for t(4;14)(p16.3q32) and t(11;14)(q13q32) (LSI® IgH/FGFR3 and LSI® IgH/CCND1 XT, Vysis, Downers Grove, IL) were used.



A A proliferation rate more than 10% in plasma cells of PM is associated with a shorter survival. Double staining for CD138/Mib1.

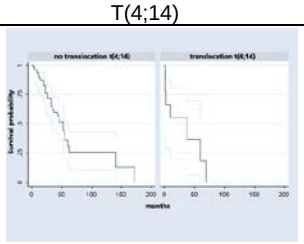


B FISH result out of the investigated series, showing a translocation for t(11;14) (*left*) indicated as a yellow dot and a low grade amplification for CCDND1 (*right*). For the latter, an additional centromer probe for chromosome 11 (*ocean blue*) was introduced (Chr11:CCND1 > 2).

Results

- **The *immature subgroup* had a significantly *poorer prognosis* (mean overall survival of 21.6 months) than the mature subgroup with a mean OS of 39.7 months ($p < 0.001$).**
- **A *high proliferation rate* (Mib1expression > 10%) was associated with a *worse outcome* (28.4 months mean survival) compared to those with a proliferation index of less than 10% (mean survival 48.5 months) ($p = 0.002$).**
- **Due to the different fixation and decalcification protocols used over the years in the different institutes, only part of the biopsies were suitable for FISH: in 77/119 (65.5%) of patients for t(4;14) and in 67/119 (53.6%) for t(11;14). The prevalence of t(4;14) was 11.7% and of the t(11;14) 20.9%.**
- **The *t(4;14)* was associated with a *statistically significantly shorter survival propability* ($p_{log\ rank} = 0.004$); On the other hand *t(11;14)* was not associated with survival in univariate analysis, in accordance with the IHC results for CyclinD1 ($p_{log\ rank} = 0.49$).**
- **A *strong CyclinD1* expression was either associated with a *t(11;14)* or a *low amplification* of the CCND1 gene.**

T(4;14)



C The t(4;14) is associated with a statistically significantly shorter survival.

Conclusion

- ***The t(4;14) and t(11;14) stratifiy mature PM into different survival groups.***
- ***Different molecular alterations*, like translocation and low grade amplification lead to an *overexpression of CyclinD1.***
- ***FISH analysis* is feasible on *bone marrow trephines*, however, standardized work up protocols are warranted (see poster Nr406)**
- ***Proliferation rate and maturation stage* should be routinely assessed in PM as a prognostic marker.**

Literatur

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