Spontaneous DNA damage and DDR pathways in WM and MM: implications for therapy

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Genomic instability is a hallmark of cancer, but its molecular basis is still under investigation. Waldenström Macroglobulinemia (MW) and Multiple Myeloma (MM) are hematologic malignancies that share many clinical and pathological features, and exhibit significant genomic instability. The DNA damage response (DDR) pathway has evolved to protect cells from both endogenous and exogenous sources of DNA damage and its role has been implicated in the disease process and activity of chemotherapy. However, the occurrence of endogenous DNA damage and the efficiency of DDR mechanism in MM and WM remain unclear. In the present study, we evaluated the role of DDR in the WM and MM and its impact on the success of chemotherapy. Intrinsic and drug-induced DNA damage were measured in WM cells lines (BCMW-1, MWCL-1) and WM patients (N=4), in MM cell lines (OPM2, RPMI8226, U266, MM1S), newly diagnosed MM patients (N=10) and paired normal peripheral mononuclear cells (PBMCs) from all patients, by using single cell electrophoresis (Comet assay) under neutral and alkaline conditions and immunocytochemistry for γH2Ax, Ku70/80, Rad51. B-cell lines Daudi, Raji, Mec1 were used as positive controls, while PBMCs from healthy donors (N=10), normal B lymphoblasts, HEEC and normal skin fibroblasts (BJ, GM05756) were used as normal controls. The levels of interstrand crosslinks(ICLs) were evaluated by Southern blot analysis. The efficiency of the two main DNA repair mechanisms, homologous recombination (HR) and Non-Homologous End Joining(NHEJ) was evaluated by using an integrated plasmid based assay. In all WM and MM cell lines significantly increased spontaneous DNA damage was observed compared to normal controls(P<0.001). Neutral Comet assay showed increased presence of DSBs in both hematologic malignancies compared to normal controls and in B-cell lymphocytes. Similarly, WM and MM patients showed significantly higher DNA damage levels compared to healthy donors. Interestingly, PBMCs from both WM and MM patients exhibited increased γH2Ax foci compared to PBMCs from healthy donors. These results were confirmed by performing immunobloting for γH2Ax, RPA, 53BP1, while immunocytochemistry showed co-localization of 53BP1 with γH2Ax. We further evaluated the different types of spontaneous DNA damage by evaluating the levels of abasic sites, DSBs and ICLs. Furthermore, we investigated the levels of cellular oxidative stress levels in MM and MW and found significant higher levels (up to 3 fold) in all human WM and MM cell lines and primary cells. Finally, the HR and NHEJ activity was elevated in MM cells lines compared

to normal fibroblasts, while WM cells exhibited lower NHEJ capacity. We further confirmed these results by evaluating the DDR and DNA repair kinetics after melphalan-induced DNA damage and also, in combination with SCR7, a NHEJ inhibitor of ligase IV. We found that inhibition of NHEJ synergizes with melphalan, enhancing its sensitivity in both WM and MM cells. Here we report, that WM and MM cells have increased levels of spontenous DNA damage. NHEJ exhibits an aberrant capacity in WM cells, suggesting a potential role of this error-prone repair pathway in genomic heterogeneity of the disease and in therapeutic interventions directed at augmenting the DNA damage.