Press Release

Double honorary distinction for the collaboration of the National Hellenic Research Foundation with the Harvard University Medical School

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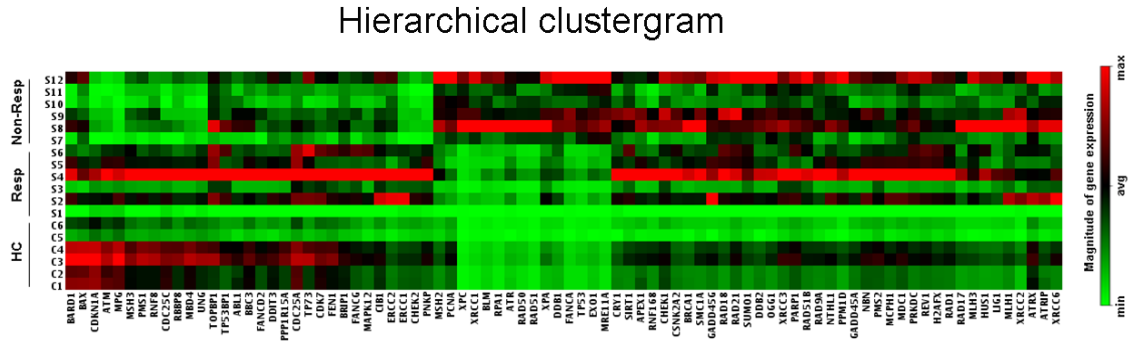

Within the framework of the 44th Annual Panhellenic Medical Conference, which took place in Athens from 9 to 12 May 2018, the research team of **Dr. Vassilis L. Souliotis**, Researcher A' at the Institute of Biology, Pharmaceutical Chemistry and Biotechnology of the National Hellenic Research Foundation, in collaboration with **Dr. Maria Gkotzamanidou**, MD, PhD (Dana-Farber Cancer Institute, Harvard Medical School and National and Kapodistrian University of Athens, School of Medicine) achieved a double honorary distinction.

The first distinction concerns the awarding of the **1st Prize of Applied Research** for their study on the enhancement of cytotoxicity of classical therapeutic agents for the treatment of multiple myeloma, by co-administration of immediate (inhibitors of DNA repair) or indirect (histone deacetylase inhibitors) modulators of DNA damage response network. The results of this work suggest that the development of **new combination protocols** offers a promising strategy toward treatment of multiple myeloma and improvement of the already used regimens.

The second honorary distinction concerns the awarding of the **"Sotiris Papastamatis" Prize** for their study on the role of the histone deacetylase HDAC8 (a member of Class I histone deacetylases) in multiple myeloma. The results demonstrate an impact of aberrant epigenome on DNA integrity through connection between HDAC8 and the DNA damage response network, and provide insights into the effect of HDAC8 on cellular growth and survival, that may have **therapeutic implications in multiple myeloma**.

1st Prize of Applied Research

Combination treatment of DNA damaging drugs and DNA repair modifiers offers a promising strategy toward improvement of existing anti-myeloma regimens

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Abstract

Objective: DNA repair activity of malignant cells affects the therapeutic outcome and patients' survival. Herein, we investigated the mechanistic basis of the link between the DNA repair efficiency and response to anti-myeloma therapy.

Methods: We studied bone marrow plasma cells (BMPCs) and peripheral blood mononuclear cells (PBMCs) of 26 unselected newly diagnosed multiple myeloma (MM) patients (12F/14M; median age: 60 years, range: 42-66) who responded (n=17) or did not respond (n=9) to subsequent melphalan therapy. PBMCs from healthy controls (HC; n=25) were studied in parallel. Cells were ex vivo treated with melphalan alone and in combination with direct (the DNA repair inhibitor SCR7) or indirect (the histone deacetylase inhibitor panobinostat) modifiers of DNA repair and the extent of DNA repair activity, apoptosis rates and the local chromatin condensation were evaluated. The expression of a focused panel of 84 genes involved in DNA damage response pathways (ATM/ATR signaling, DNA repair, cell cycle regulation, apoptosis) was also examined. Western blot was used for the analysis of DNA repair proteins.

Results: Both BMPCs and PBMCs from responders to melphalan therapy showed more condensed chromatin structure and slower rates of DNA repair ($P<0.0022$) compared to non-responders. Apoptosis rates of both BMPCs and PBMCs were inversely correlated with individual DNA repair efficiency, being higher in responders' cells compared to those of non-responders ($P=0.0011$). PBMCs from MM patients showed higher looseness of chromatin structure, increased DNA repair activity and higher apoptosis rates compared to HC. Microarray analyses of untreated PBMCs showed that responders are characterized by significant upregulation of ATR, BLM, DDB1, EXO1, FANCA, MRE11A, MSH2, PCNA, RAD50, RAD51, RPA1, XPA, XPC, XRCC1 and TP53 genes and downregulation of ATM, CDC25C, CDKN1A, CHEK2, ERCC1, MPG, PNKP and UNG genes compared to non-responders ($P<0.001$), suggesting that perturbation in the molecular components of DNA damage response pathways plays an important role in the therapeutic action of the genotoxic drugs. Interestingly, co-treatment of cells with

melphalan and the DNA repair inhibitor SCR7 significantly reduced the rates of DNA repair and increased melphalan sensitivity of both BMPCs and PBMCs. Moreover, co-treatment of BMPCs with melphalan and the HDACi panobinostat resulted in hyperacetylation of histone H4, increased DNA damage burden and higher apoptosis rates. On the contrary, the co-treatment of PBMCs from the same patients with melphalan in the presence of panobinostat did not significantly alter the melphalan-induced DNA damage burden and the apoptosis rates. Interestingly, by using Western blot analysis, we found that treatment with panobinostat decreased the levels of critical DNA repair proteins (Rad50, Mre11, Ku70, Ku86, DNA-PKcs) in malignant BMPCs, while it did not suppressed these DNA repair proteins in PBMCs. These findings can explain, in part, the selectivity of this HDACi in causing increased DNA damage burden and cell death in malignant BMPCs at concentrations that cause no cell death in PBMCs from the same individuals.

Conclusion: The enhancement of melphalan cytotoxicity by direct (DNA repair inhibitors) or indirect (HDACi) DNA repair modifiers offers a promising strategy toward treatment of MM and improvement of already used regimens.

"Sotiris Papastamatis" Prize

HDAC8 is a new epigenetic target in the treatment of multiple myeloma by affecting on DNA repair pathways and cellular transcription dynamics

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Abstract

Aims: To date, both clinical and preclinical studies have confirmed that MM is vulnerable to epigenetic intervention, with histone deacetylase enzymes (HDAC) emerging as the most promising epigenetic targets. We aim to investigate the function of HDAC8 in MM biology and to evaluate its potency as therapeutic target.

Methods: Lentiviral-shRNA delivery system was used for knockdown of HDAC8 in OPM2 and U266 cells. The HDAC8 inhibitor PCI-34051 was used as chemical inhibitor. A panel of 15 antibodies was used in immunoblot analysis. Immunofluorescence staining was performed and the images were analyzed using confocal microscopy. Single-cell gel electrophoresis under neutral conditions (neutral comet assay) was performed in OPM2-HDAC8 knockdown cells with or without exposure to gamma-irradiation (IR), and in treated and untreated cells with HDAC8 inhibitor in combination to IR. Co-

immunoprecipitation assay was performed for investigation of interactions of HDAC8 after induction of DNA damage. DNA double-strand break repair (DSB/R) occurring via homologous recombination (HR) pathway was assessed using a transient direct repeat DsRED-GFP/I-SceI plasmid-based system. Expression of DNA damage response (DDR) genes was evaluated using a high-throughput PCR assay. Cellular senescence was assessed with SA-β-Galactosidase staining.

Results: We evaluated the expression of HDAC8 in 172 newly-diagnosed MM patients from the IFM myeloma dataset and observed HDAC8 overexpression as well as its significant correlation with poor survival outcome ($P<0.0015$). We further evaluated the expression of HDAC8 in HMCLs and confirmed the high expression and its cytoplasm and nuclear localization in all five MM cell lines studied. The HDAC8 depletion in two MM cell lines resulted in significant inhibition of proliferation of MM cells at 1 week, and decrease in colony formation ($P<.001$). The combination of HDAC8 inhibitor with melphalan or bendamustine enhanced the anti-MM effects of the genotoxic agents (all $P<0.01$). Interestingly, U266 cells with HDAC8 depletion exhibited increased levels of markers of DNA damage. Moreover, in consistence with this observation HDAC8 knock-down led to decreased HR activity and decreased repair of DSBs after IR. Similar results were also obtained with HDAC8 inhibitor. The HDAC8 protein co-localized and co-immunoprecipitated with p53 after IR and with Scm3, member of cohesin. Finally, the depletion of HDAC8 resulted in the higher prevalence of senescence associated with β-Gal-positive cells 3 weeks post transduction.

Summary/Conclusion: Our results demonstrate an impact of aberrant epigenome on DNA integrity through connection between HDAC8 and the DNA damage response network, and provide insights into the effect of HDAC8 on cellular growth and survival with potent therapeutic implications in MM.

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