[ABSTRACT WM3.12]

Resveratrol exerts antiproliferative effect and induces apoptosis in Waldenström's Macroglobulinemia

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Background: Resveratrol (3,4',5-trihydroxystilbene) is a polyphenolic natural product, synthesized by a wide variety of plant species including grapes. It has gained considerable attention because of its anti-cancer properties, as demonstrated in solid and haematological malignancies, including multiple myeloma. We therefore examined Resveratrol for its anti-tumor activity in Waldenström's Macroglobulinemia (WM). Methods: We examined the effect of increasing concentrations of resveratrol (5-80 mM) on WM cell lines (BCWM.1), IgM secreting low-grade lymphoma cell lines (WM-WSU, MEC-1, RL), primary CD19+ WM cells and bone marrow stromal cells (BMSCs) isolated from bone marrow of patients with WM, after appropriate informed consent. [3H]-thymidine uptake and calcein-AM assay were used to evaluate the effect of resveratrol on proliferation and cytotoxicity respectively. Apoptosis and cell cycle analysis were investigated at 24h by flow cytometry using Annexin V-propidium iodide (PI) staining and PI-staining respectively. Apoptotic and cell signaling pathways targeted by resveratrol were investigated by Western Blot at 24 h and 6 h respectively. Since BMCSc confer growth and resistance to conventional treatments, we also tested the effect of resveratrol on WM cells co-cultured with BMSCs. Results: Resveratrol induced significant cytotoxicity and inhibition of DNA synthesis at 24 and 48 h on BCWM.1 with an IC50 of 10-20mM. Similar data was obtained with primary CD19+ WM cells. In contrast, resveratrol did not trigger significant reduction of proliferation of peripheral blood mononuclear cells isolated from healthy donors. Importantly, it induced apoptosis in BCWM.1 and primary CD19+ WM cells, as demonstrated by flow cytometry. Dose-dependent apoptosis at 24h with induction of caspases 3, 8, 9 and PARP cleavage was also observed, including reduction of Mcl-1 and increase of p53. In parallel, resveratrol caused accumulation of BCWM.1 in sub-G1 phase. To better elucidate the mechanism of action of resveratrol in WM, we next examined downstream molecules and observed that resveratrol inhibited Akt phosphorylation in BCWM.1 cells in a dose-dependent manner. Phosphorylation of GSK3a/b, downstream target protein of Akt, was also markedly inhibited, as well pERK and pAKT. Adherence of BCWM.1 cells to BMSCs triggered increased [3H] thymidine uptake, and resveratrol inhibited this up-regulation in a dose-dependent manner. Conclusion: These in vitro data demonstrated that resveratrol has significant antitumor activity in WM, providing the framework for clinical trials in WM patients.