



HSP90 INHIBITOR STA-9090 DOWNREGULATES EXPRESSION OF HSP90 CLIENT PROTEIN WT1 IN MYELOID LEUKEMIA CELLS

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ABSTRACT

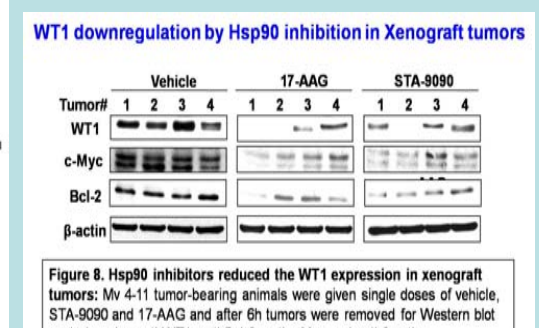
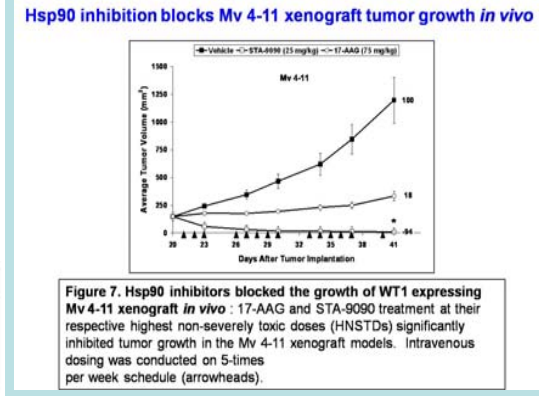
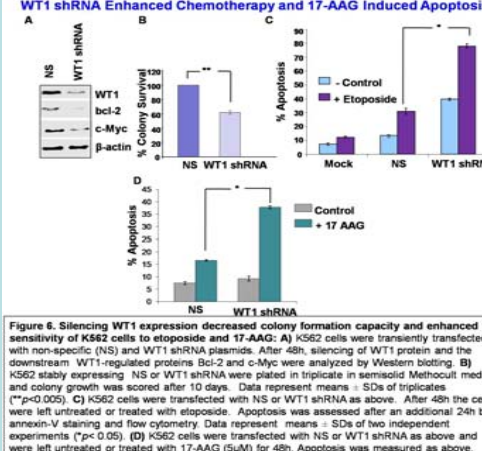
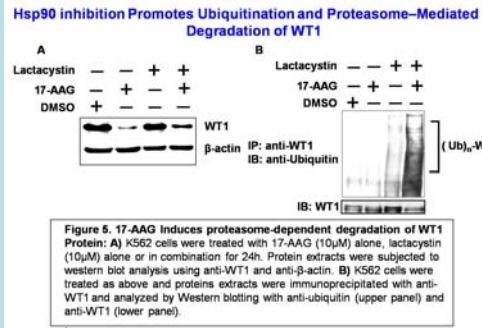
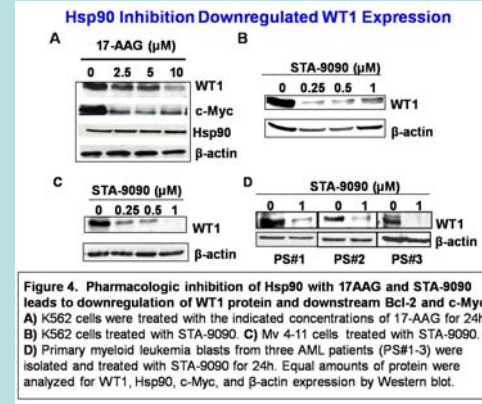
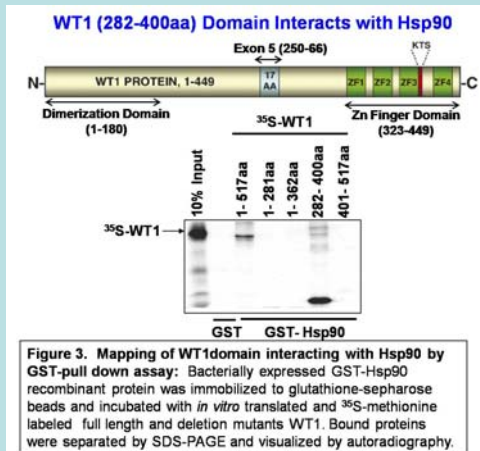
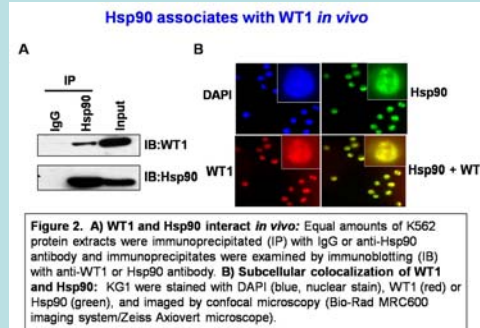
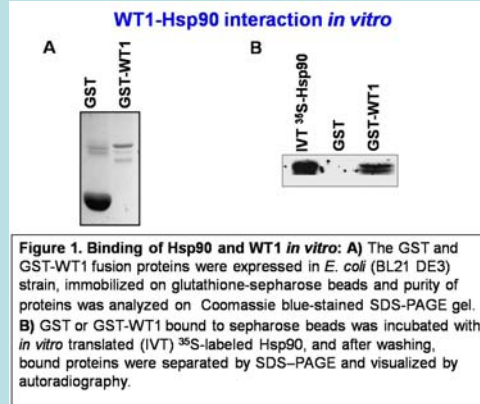
Background: Aberrant expression of pro-survival and anti-apoptotic proteins plays an essential role in myeloid leukemia cell survival and mediates resistance to chemotherapy. The Wilms' tumor 1 (WT1) protein is a transcription factor that has been reported to be over-expressed in acute myeloid leukemia (AML). Over-expression of WT1 has been associated with relapse and shortened disease-free survival in AML patients. Heat shock protein (Hsp90) is a molecular chaperone involved in maintaining the stability and function of many key proteins which are deregulated in cancers. This work was aimed at elucidating the role of Hsp90 in the regulation of WT1 expression in myeloid leukemia.

Methods: The WT1-Hsp90 interaction was analyzed by co-immunoprecipitation, confocal microscopy and glutathione S-transferase (GST)-pull down assays. The effect of Hsp90 inhibition on WT1 protein expression was determined by Western blotting. The role of WT1 in the growth and survival of leukemia cells was demonstrated *in vitro* by short-hairpin RNA (shRNA) mediated silencing of WT1, and confirmed *in vivo* in xenograft tumor models by treatment with Hsp90 inhibitors.

Results: Here we report that WT1 is a client protein of Hsp90. WT1 was observed to co-localize with Hsp90 in the nuclei of K562 leukemia cells and endogenous WT1 was co-immunoprecipitated with an anti-Hsp90 antibody. Pharmacological inhibition of Hsp90 by the second-generation Hsp90 inhibitor, STA-9090 (a novel resorcinol-containing compound) reduced the expression of WT1 protein in a dose-dependent manner in myeloid leukemia cell lines. Consistent with this, WT1 downregulation by Hsp90 inhibition also resulted in reduced expression of the WT1 target protein c-Myc. STA-9090 more potently inhibited WT1 protein expression than the first-generation Hsp90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17-AAG). WT1 down-regulation by STA-9090 was also observed in primary myeloid leukemia blasts isolated from AML patients. Hsp90 inhibition resulted in ubiquitination and subsequent proteasome-dependent degradation of WT1. Furthermore, silencing of WT1 with shRNA potentiated apoptosis by chemotherapeutic agents and further sensitized leukemia cells to Hsp90 inhibitors. Inhibition of Hsp90 blocked *in vivo* tumor growth in a xenograft tumor study using leukemia cells expressing WT1. Consistent with our observations *in vitro*, STA-9090 displayed significantly greater anti-tumor activity *in vivo* than 17-AAG.

Conclusions: WT1 is a substrate for Hsp90 and this WT1-Hsp90 interaction is crucial for leukemia blast survival. As there is no established therapy that durably inhibits WT1 oncogenic functions, targeting WT1 expression by Hsp90 inhibitors like STA-9090 may offer new strategies to limit the survival promoting effects of WT1 in myeloid leukemias.

RESULTS



SUMMARY

- Hsp90 interacts with WT1 in leukemia cells and stabilizes WT1 expression.
- Pharmacologic inhibition of Hsp90 function leads to downregulation of WT1 protein.
- 17-AAG induces ubiquitination and proteasome-mediated WT1 protein degradation.
- Hsp90 inhibitors 17AAG and STA-9090 significantly reduced the growth of myeloid leukemia xenografts *in vivo* and effectively downregulated the expression of WT1 and its downstream target proteins c-Myc and Bcl-2 in xenograft tumors.