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“Ubiquitin-Proteasome System stress sensitizes ovarian cancer cells to proteasome inhibitor-induced apoptosis”

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Background: Despite evidence for dysregulation of the Ubiquitin-Proteasome System (UPS) with tumorigenesis, tumor progression and drug resistance, the relationship between proteasome expression and cancer progression is not fully understood. Since better therapies are urgently needed for ovarian cancer, we examined the sensitivity of ovarian cancer cells to proteasomal inhibition and the mechanistic basis for sensitization of cancer cells to proteasomal inhibition.

Design: Expression of proteasome and poly-ubiquitinated proteins was determined by immunoblot and immunocytochemical (ICC) analysis in ovarian cancer cell lines and human specimens of ovarian cancer. Proteasome inhibitors were used to assess the effect of proteasomal inhibition on cultured cell lines and xenografts established with ES-2, an aggressive, chemoresistant ovarian cancer cell line.

Results: Immunoblot and ICC analysis show significant up-regulation of proteasome expression in ovarian cancer cells and in ovarian tumors versus benign and normal epithelial cells. Despite higher levels of proteasome subunits, ovarian cancer cells and tumors show increased accumulation of poly-ubiquitinated client proteins. This suggests that the upregulation of the proteasome levels in ovarian cancer cells may be insufficient to meet the increased demand for proteasome function and suggests UPS stress. Consistent with this hypothesis, ovarian cancer cells are more sensitive to proteasomal inhibition than immortalized ovarian surface epithelial cells. We also found that the sensitivity to proteasomal inhibition relates directly to their proliferation rate and UPS stress, but not the levels of proteasome, suggesting that higher metabolic demand in rapidly proliferating cells lead to UPS stress. The sensitivity of ovarian cancer cells to proteasomal inhibition is independent of p53 status. We show that proteasomal inhibition leads to accumulation of cyclin-dependent kinases (CDK), p21^{WAF1} and p27^{Kip1}, concomitant with G2/M arrest followed by apoptosis. This suggests a role for these CDKs in blocking the G2/M transition of the cells after proteasomal inhibition and subsequent cell death. We also show that while cell death is caspase-dependent, caspase-3 is not entirely responsible for the apoptotic process upon proteasomal inhibition. This is consistent with a study showing the activation of caspase 3, 8, and 12 with proteasome inhibition. ICC analysis of cells treated with proteasome inhibitors show the accumulation of ubiquitinated protein in aggresome-like structures, indicating that multiple protein metabolic pathways are affected by proteasome inhibition. Finally by using our xenograft model we show that PS-341 treatment significantly reduces tumor growth and enhances survival of mice bearing tumors (P<0.001).

Conclusion: Ovarian carcinoma exhibits elevated stress of the UPS system. Our data provide an insight for understanding the mechanistic basis of sensitivity of ovarian cancer cells to proteasomal inhibition. Finally, we provide a strong preclinical evidence for using proteasome inhibitors such as PS-341 as a chemotherapeutic agent for ovarian cancer.