

ABSTRACT

Heat shock protein 90 (HSP90), which is implicated in post-translational folding, stability, and maturation of proteins, controls several key cell cycle regulators. Thus, the hypothesis was raised that geldanamycin, a specific and potent inhibitor of HSP90 function, may have pronounced effects on cell cycle progression. The objective of this study was to test this hypothesis in normal and cancer cells of human origin.

The experiments performed on human lymphocytes mitogenically stimulated by phytohemagglutinin (PHA) indicated that 100 nM or 150 nM geldanamycin induces transition of cells to the G0 state of cell cycle. This was documented utilizing acridine orange, a metachromatic dye which differentially stains DNA versus RNA. The same experimental protocol allowed demonstration that geldanamycin is a potent inducer of apoptosis in PHA-activated cells. Importantly, both the block in G0 and induction of apoptosis were reversible and returned to control values upon removal of geldanamycin. Similar conclusions were reached when cell number in cultures was analyzed, excluding the possibility that a relevant fraction of cells was disintegrated during the incubation period.

Experiments on Jurkat line of acute T-cell leukemia were performed next. Jurkat cells were used here as a model system in which the cytostatic and cytotoxic properties of geldanamycin on cancer cells can be tested. Initial experiments determined the time course and concentration-dependence of geldanamycin-induced alterations in cell cycle distribution and apoptosis. In contrast to human lymphocytes, geldanamycin did not induce G0 arrest in Jurkat cells, but inhibited them initially in the G2 phase, and at later time points in the G1 phase. The G2 was distinguished from mitosis by the absence of phosphorylation of histone H3, a specific marker of mitotic cells. The inhibition of Jurkat cells in G1 was linked to a decrease in phosphorylation of retinoblastoma protein. Finally, the exposure of Jurkat cells to geldanamycin resulted in induction of apoptosis, predominantly in cells being arrested in G1 and G2/M phases of the cell cycle.

Finally, to address the possibility that stimulation of nuclear factor kappa-B (NF- κ B), downstream of HSP90, modulates the effects of geldanamycin on cancer cells, Jurkat I κ B α M line was employed. These cells cannot activate their NF- κ B-mediated responses

because of the mutation in its inhibitory protein, I κ B. In the absence of functional NF- κ B, geldanamycin-mediated induction of apoptosis and loss of cycling cells were markedly higher than when NF- κ B was functional. These results were further corroborated by experiments in which parthenolide, a plant-derived inhibitor of NF- κ B was employed. Geldanamycin-treated Jurkat cells responded to the parthenolide challenge by partial arrest in S phase and increased cell death by apoptosis.

In conclusion, inhibition of HSP90 by geldanamycin blocks cell cycle progression and induces apoptosis of Jurkat cells, and NF- κ B mediates these effects. The newly identified network of interactions may facilitate understanding of the mechanism of cytostatic and cytotoxic action of geldanamycin derivatives used currently in clinical trials.