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Plumbagin induces G2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells

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Abstract

This study is the first to investigate the anticancer effect of plumbagin in human breast cancer cells. Plumbagin exhibited cell proliferation inhibition by inducing cells to undergo G2/M arrest and autophagic cell death. Blockade of the cell cycle was associated with increased p21/WAF1 expression and Chk2 activation, and reduced amounts of cyclinB1, cyclinA, Cdc2 and Cdc25C. Plumbagin also reduced Cdc2 function by increasing the association of p21/WAF1/Cdc2 complex and the levels of inactivated phospho-Cdc2 and phospho-Cdc25C by Chk2 activation. Plumbagin triggered autophagic cell death, but not, predominantly, apoptosis. Pretreatment of cells with autophagy inhibitor bafilomycin suppressed plumbagin-mediated cell death. We also found that plumbagin inhibited survival signaling through the PI3K (phosphatidylinositol 3-kinase)/AKT signaling pathway by blocking the activation of AKT and downstream targets, including the mammalian target of rapamycin (mTOR), forkhead transcription factors (FKHR) and glycogen synthase kinase-3beta (GSK). Phosphorylation of both of mTOR's downstream targets, p70 ribosomal protein S6 kinase (p70S6 kinase) and 4E-BP1, was also diminished. Overexpression of AKT by AKT cDNA transfection decreased plumbagin-mediated autophagic cell death, whereas reduction of AKT expression by siRNA potentiated plumbagin's effect, supporting inhibition of AKT beneficial to autophagy. Furthermore, suppression of AKT by plumbagin enhanced the activation of Chk2, resulting in increased inactive phosphorylation of Cdc25C and Cdc2. Further investigation revealed that plumbagin's inhibition of cell growth was also evident in a nude mice model. Taken together, these results imply a critical role for AKT inhibition in plumbagin-induced G2/M arrest and autophagy of human breast cancer cells.

Keywords: Plumbagin, autophagy, AKT