S179D Prolactin (PRL) primarily uses the extrinsic pathway and MAPkinase signaling to induce apoptosis in human endothelial cells.

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S179D Prolactin (S179D PRL) is a molecular mimic of phosphorylated human PRL. In previous studies, we have demonstrated that this molecule inhibits the growth of prostate tumors *in vivo*. Partly, this is brought about by blockade of the autocrine and growth- promoting unmodified PRL produced by prostate epithelial cells, and partly this is likely due to the anti-angiogenic effects of S179D PRL. S179D PRL is a potent anti-angiogenic compound *in vitro* and *in vivo*. In this study, we examined apoptosis in human endothelial cells, using procaspase-8 as a marker of the extrinsic pathway, and cytochrome C release as a marker of the intrinsic pathway. Both pathways converge at caspase-3. which cleaves DNA fragmentation factor (DFF45). A 3-day incubation in 50 ng/ml S179D PRL quadrupled the early apoptotic cells; this effect was doubled at 100 ng/ml and maximal at 500 ng/ml. DFF45 and pro-caspase 8 cleavage were detectable at 100 ng/ml. Cytochrome C. however, was unaffected until 500 ng/ml. p21 increased at 100 ng/ml, whereas a change in p53 required both triple the time and 500 ng/ml. p21 promoter activity was maximal at 50 ng/ml, whereas 500 ng/ml were required to see a significant change in the Bax promoter (a measure of p53 activity). Since S179D PRL and bFGF both activate ERK. we examined the effect of S179D PRL on bFGF-induced ERK signaling. As previously shown, S179D PRL blocked ERK phosphorylation in response to bFGF, but in addition, continued co-incubation showed a delayed and prolonged activation of ERK. PD98059 inhibited this delayed activation of ERK and effects of S179D PRL on all measures except p53 protein, or activity of the Bax promoter. We conclude that low doses of S179D PRL block bFGF-induced ERK signaling and yet use ERK in a different time frame to elevate p21. and activate the extrinsic pathway. Longer incubations and higher concentrations, however, additionally activate the intrinsic pathway using an alternate intracellular signal.

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