

# PEP005, a novel ingenol angelate mediates apoptosis in human cancer cell lines by activation of p38 and MAPK pathways, via a PKC-dependent mechanism

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**Background:** PEP005 (3-ingenyl angelate) is a novel agent extracted from *Euphorbia peplus* that activates PKC $\delta$ , resulting in cytotoxic (Can Res 2004, 64:2833) and pro-apoptotic effects (Blood 2005, Epub). Currently, PEP005 is being developed as a topical treatment for actinic keratoses and basal cell skin carcinoma and as a systemic treatment for leukemia by an Australian company, Peplin Ltd. Cytotoxicity of PEP005 was assessed in several human solid tumor cell lines (KA Benhadji *et al.* NCI-AACR-EORTC 2005). Although, the precise mechanism of action of PEP005 remains unknown, *in vitro* and *in vivo* laboratory studies have shown that two separate mechanisms exist and that both may require activation of PKCs. At relatively high concentrations of PEP005 (100-200 $\mu$ M), mitochondrial disruption, and cell death were associated with PEP005 cytotoxicity (Can Res 2004, 64:2833). At relatively low concentrations of PEP005 (1-10 nM), PKC $\delta$ -dependent apoptosis was associated with PEP005 cytotoxicity of leukemia cells (Blood 2005, Epub). The aim of our study was to further explore the molecular mechanisms of action of PEP005 in the human solid tumor models.

**Methods:** Baseline PKC-related signaling, MAPK signaling, and apoptotic pathways were determined using western blots in a panel of colon, breast, ovarian and lung cancer cells. Modulation of those pathways using PEP005 and various specific pharmacological inhibitors were assessed comparatively in PEP005 sensitive and resistant cancer cells.

**Results:** PEP005 displayed antiproliferative effects in human colon COLO205 and breast MDA435 cancer cells (IC50s: 0.01 and 2.6 $\mu$ M, respectively). Although most cancer cells used in our panel were shown to express several isoforms of PKC, no clear correlation between PKC isoform expression and sensitivity to PEP005 was detected. FACS analysis of COLO205 cells treated with PEP005 for 1, 5, 12, 24 or 48 hours showed early cell-cycle changes (G1/S block) and apoptosis induction followed by secondary necrosis after 24 hour incubation. PEP005-induced apoptosis was caspase-3 dependent but Bcl-2 and p53-independent. Total protein levels of PKC $\alpha$ , phos-PKC $\alpha$ , PKC $\delta$ , phos-PKC $\delta$ , PKC $\epsilon$ , p53 and MDM2 were compared after PEP005 treatment in COLO205 sensitive and HT29 resistant cell lines to determine the mechanism of PEP005 sensitivity. PEP005 downregulated the expression and activity of phosphorylated (active) levels of PKC $\alpha$  in a dose-dependent manner. In contrast active, phosphorylated PKC $\delta$  and p38 levels were highly (7-10 fold) upregulated by PEP005 in COLO205 sensitive cells but only slightly (2-3 fold) upregulated in HT29 resistant cancer cells. Activation of p38 by PEP005 was strongly dependent on PKC $\delta$  since cell pretreatment with rottlerin, a specific inhibitor of PKC $\delta$ , blocked this activation. Activation of PKC $\delta$  was followed by its translocation from cytosol to nucleus and mitochondrial membranes. Furthermore, pharmacological inhibitors suggested that MEK1/2, ERK1/2, and other MAP kinases may be involved in PEP005-PKC downstream signaling pathways.

**Conclusion:** Our results suggest that PEP005 induced caspase 3-dependent but p53- and Bcl-2-independent apoptosis. Cytotoxic effects were related to the activation of PKC $\delta$  and downregulation of PKC $\alpha$  and also involve MAPK signaling pathways. Our results are consistent with those obtained in leukemic cell lines. This study supports ongoing efforts targeting PKC isoforms in cancer therapy with PEP005 alone and in combination with cytotoxic agents.