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Nucleolin targeting oncolytic peptide for treatment of cancer

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New targeted cancer therapies under development deliver cytotoxic molecules to cancer cells via specific binding of ligands to molecules or receptors that are over-expressed on the surface of cancer cells. Nucleolin is a protein that is over-expressed on the surface and cytoplasm of cancer and endothelial cells compared to normal cells. EP-302 consists of a nucleolin binding moiety conjugated to a novel lytic peptide called CLIP-71 to target and destroy cells that express nucleolin on their surfaces. It was tested *in vitro* in 20 human cancer cell lines (including breast, endometrial, ovarian, pancreatic, prostate, colon, lung and hematological malignancies). Nucleolin negative HS27 human fibroblast and 3T3 cells were used as negative controls. Cells were cultured in the presence of various concentrations (0.0001-100 μ M) of EP-302 or unconjugated CLIP-71 for 0.25 to 24h. Hemolytic effects of EP-302 on human red blood cells, plasma stability and tolerability in mice were also tested. Viability was measured by formazan conversion assays. In addition, specific binding to cell surface nucleolin was determined by immunohistochemistry in cell lines western blot analysis from membrane preparations and tumor sections. *In vivo* efficacy of EP-302 was conducted in female and male nude mice implanted with MDA-MB-435S or PC-3 cells. Treatment groups were: saline controls, 0.02, 0.2 and 1 mg/kg (PC-3 xenografts N=16 per group) and 0.2 and 1 mg/kg (MDAMB-435 xenografts, N=10 per group). Mice were treated weekly for 3 weeks via a bolus injection into the lateral tail vein. Results show that EP-302 caused a dose-dependent cell killing after 24h incubation in MDA-MB-435S (IC₅₀ - 1.8 μ M) and PC-3 (IC₅₀ - 3.3 μ M) cells and 53 μ M in nucleolin negative HS27 and 3 T3 cells. Sixteen human cancer cell lines had IC₅₀ values of 0.5-6.4 μ M. Nucleolin binding peptide F3 inhibited the cytotoxicity of EP-302 indicating that the cytotoxic effects of EP-302 are mediated via binding to nucleolin. There was no hemolytic activity at concentrations <1000 μ M and plasma stability studies showed that > 60% bioactivity was present after 2 h of incubation. Studies with fluorescein-labeled EP-302 demonstrated specific binding and cell destruction within 30 minutes. The maximal tolerated dose in nude mice was 8 mg/kg EP-302 after daily repeated injections for 5 days. *In vivo* efficacy studies resulted in significant ($p < 0.0035$) tumor regression for PC-3 xenograft at doses of 0.02, 0.2 and 1 mg/kg and increased survival of the mice. Tumor regression was also found in the MDA-MB-435S xenograft ($p < 0.0001$) at all doses tested. Histological evaluation of treated tumors showed that cell killing was primarily by necrosis. These results indicate that EP-302 selectively targets and kills only cancer cells that have surface nucleolin. They demonstrate that EP-302 is a potential therapy for cancers in humans.