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Induction of Melanoma Cell Apoptosis by Fas Ligand (21-May-2001)

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The long-term goal of this work is to establish optimal conditions to promote apoptosis of tumors at accessible sites, thereby providing a source of antigen to promote anti-tumor immune responses. For this study, we examined the capability to induce apoptosis of canine melanoma cells *in vitro* by overexpression of Fas ligand (FasL). FasL was expressed in five canine melanoma cell lines by infection with a replication-deficient adenovirus encoding murine FasL, or by cationic liposome-mediated transfection of a plasmid encoding human FasL. Four of the five cell lines underwent extensive apoptosis. One cell line was resistant to apoptosis, despite detectable levels of FasL expression as determined by its ability to kill Fas-bearing L1210-Fas target cells. Using this resistant cell line, we determined there was a log-linear relationship between FasL expression and multiplicity of infection (m.o.i.) to >1,000 viral particles per cell. Resistance to Fas-mediated killing could thus be due to loss of Fas expression by the tumor cells, or to inhibition of signaling pathways that operate downstream of Fas. Fas mRNA was expressed by each of the four susceptible melanoma cell lines, but not by the resistant cell line. In addition, downstream signaling events may contribute to the resistant phenotype. It has been reported previously that susceptibility to FasL-mediated apoptosis requires at least one wild type copy of the PTEN tumor suppressor gene. Our results show that the resistant cell line also lacked expression of PTEN mRNA and protein, but so did two of the susceptible cell lines. However, the resistant cells also lacked mRNA for p16, and had markedly reduced levels of p53 and p21. Next, we assessed the safety of FasL administration *in vivo* to tumor-bearing dogs. FasL (600 µg), mixed with 1 mg of cationic liposomes, was administered by direct intratumoral injection to five dogs with oral or facial tumors with high metastatic potential (4 malignant melanoma, 1 osteosarcoma). No adverse events were observed over the course of 7 days, after which dogs were provided standard therapy as indicated for their tumor (surgery or radiation). Three of five tumors showed measurable regression at day 7. Cells isolated from the two tumors showing the most robust responses had detectable Fas expression, whereas those isolated from the tumor showing the weakest response, and those isolated from a tumor with no measurable response had no detectable Fas mRNA. The data suggest that overexpression of FasL is an effective means to induce apoptosis of canine melanoma cells *in vitro*, and that susceptibility may be determined by distinct genetic "fingerprints". Moreover, intratumoral gene administration appears to provide a safe route for delivery of FasL *in vivo*.