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Regulation and Function of Fas Expression in Tumor Cells *in-vivo* and *in-vitro*

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The death receptor Fas (CD95/APO-1) appears to be involved in both pathological and normal processes of the immune system, and to play a role in tumor immunity and progression. Although Fas has been studied extensively, its precise function(s) and the regulation of its expression in tumors are not fully understood. Our lab reported that *in vitro* cultured, low Fas-expressing tumors underwent massive yet reversible up-regulation of Fas expression when injected into mice (Rosen et al., 2000). The exact regulation of Fas expression *in-vivo*, host and/or tumor factors involved are unknown. The present study was aimed at determining host factors involved in Fas up-regulation in tumor cells induced *in-vivo*, as well as examining the relationship between Fas expression and Fas activity. Fas up-regulation was accompanied by an enhanced rate of Fas-based apoptosis induce by Fas antibody, perforin-deficient CTL and by FasL trimer. Because Fas up-regulated tumor cells displayed enhanced apoptosis in response to Etoposide, increased susceptibility to apoptosis appears to be effected through the caspase pathway. Fas up-regulation was retarded when tumors were injected into immune deficient or X-irradiated mice suggesting involvement of the immune system. Compatible with this observation was the finding that Fas up-regulation could be reproduced *in-vitro* when tumor cells were co-cultured with immune spleen or peritoneal exudates cells. Fas-up regulation also occurred when low Fas expressing tumors were cultured in diluted ascitic fluids collected from tumor bearing mice. The results suggest that Fas up-regulation in tumor cells involves a soluble factors produced (e.g. nitric oxide or cytokines) and induced by the immune system. Experiments with the nitric oxide (NO) donor SNAP suggest that NO is involved in regulating Fas expression *in-vivo*.

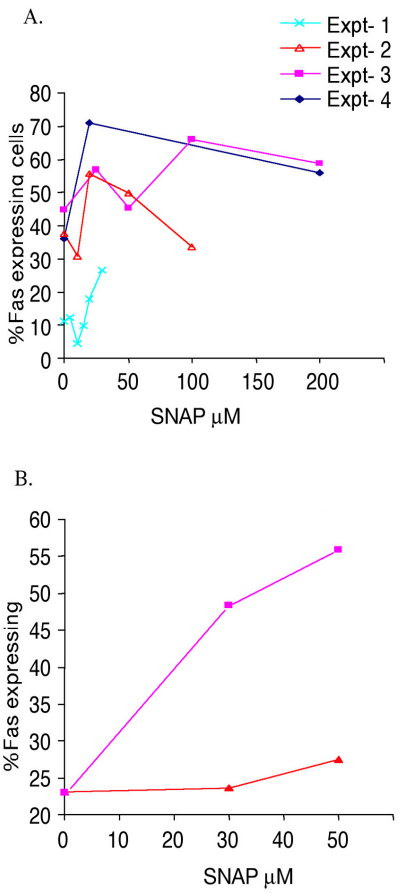

Fig. 1 The effect of the NO donor SNAP on Fas expression

A. *Low Fas expressing (cultured) RMA-S tumor cells were exposed to 0-200 μM of the NO donor SNAP, added once at the beginning of the experiment. After 17h incubation, cells were analyzed for Fas expression, by FACS.*

B. *RMA-S tumor cells were incubated in the presence of SNAP (0-50 μM for 23h), added either once (■)at the beginning of the incubation or every 5-7h during the incubation (▲). The Fig represents one of 5 experiments.*

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