549

STAT5a is activated in human breast cancers and associates with the p85 subunit of PI3 kinase

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Mouse model studies demonstrate that activated Stat5a acts as a survival factor for normal and malignant mammary epithelial cells. Like other STAT (Signal Transducer and Activator of Transcription) family members, Stat5a is activated through tyrosine phosphorylation in the cytoplasm and translocated to the nucleus where it can act as a transcription factor. To determine if activated Stat5a could be a significant survival factor and possible therapeutic target for human breast cancers, the frequency of Stat5a activation was established in a series of 83 randomly selected primary breast cancers. Techniques included immunohistochemistry and immunoprecipitation/Western blot assays. Statistical analyses were performed to determine if the presence of activated Stat5a was correlated with tumor grade, pre/post menopausal status, lymph node metastases, ploidy, S phase, apoptotic index, Estrogen Receptor alpha, Progesterone Receptor, ErbB2, Cyclin D1, p21, p27, Stat5b and Stat3. Overall, 75% of the breast cancers examined demonstrated Stat5a activation. There was no direct correlation between the presence/absence of nuclear localized Stat5a and nuclear localized Stat5b or Stat3. Stat5b was localized cytoplasmically more frequently in comparison to the primarily nuclear localization of Stat5a. Stat3 was both nuclear and cytoplasmically localized. Stat5a nuclear staining status (negative/positive) was inversely related to Progesterone Receptor status (negative/positive) at a statistical significance level of p=0.02. There was a weaker positive association with differentiation status (p=0.03). In some cell systems Stat5a increases cell survival by up-regulating bcl-x transcription. However, in mouse mammary epithelial cells, prolactin induced Stat5a/b activation cannot up-regulate bcl-x transcription and bcl-x is transcribed at normal levels in Stat5a/b knockout mice. Experiments to identify alternative downstream survival pathways activated by Stat5a in primary human breast cancers established that Stat5a physically interacts with the p85 regulatory subunit of PI3kinase as demonstrated by co-immunoprecipitation. The results indicate that Stat5a is activated in a significant proportion of human breast cancers. The negative association with Progesterone Receptor expression is consistent with an inhibitory effect of activated Stat5a on ER alpha mediated transcription *in vivo*. Supported in part by USAMC DOD Breast Cancer Research Program BC000716.

550

Smac agonists sensitize for TRAIL- or anticancer drug-induced apoptosis and induce eradication of malignant glioma *in vivo*

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Due to intrinsic resistance of many tumors to established therapies, current attempts to improve the survival of cancer patients largely depend on strategies to target tumor cell resistance. Since induction of apoptosis in target cells is a key mechanism for most antitumor therapies, defects in apoptosis programs may cause resistance. "Inhibitor of Apoptosis Proteins" (IAPs) contribute to the resistance of cancers, since they are expressed at high levels in many tumors including malignant glioma. Here, we report that Smac gene transfer or cell-permeable Smac peptides sensitized various tumor cell lines *in vitro* and malignant glioma cells *in vivo* for apoptosis induced by death receptor ligation or cytotoxic drugs by antagonizing the apoptosis inhibitor XIAP. Ectopic expression of a cytosolic active form of Smac or cell-permeable Smac peptides bypassed the Bcl-2 block, which prevented the release of Smac from mitochondria. Also, Smac peptides sensitized resistant neuroblastoma cells lacking caspase-8, melanoma cells lacking Apaf-1 or patient-derived primary neuroblastoma cells ex vivo. Most importantly, Smac peptides strongly enhanced the antitumor activity of TRAIL in an intracranial malignant glioma xenograft model *in vivo* over a wide range of TRAIL concentrations. Maximal synergy of the combined treatment with Smac peptides and TRAIL was found at suboptimal concentrations of TRAIL that on their own only temporarily delayed tumor growth. Complete eradication of established tumors and survival of mice was only achieved upon combined treatment with Smac peptides and TRAIL. Importantly, administration of Smac peptides did not reverse the lack of toxicity of TRAIL for nontransformed cells and normal tissues both *in vitro* and also *in vivo*. This indicates the tumor specificity –and thus the possible safety- of the combined treatment with Smac peptides and TRAIL. Thus, Smac agonists represent novel promising cancer therapeutics to potentiate the efficacy of cytotoxic therapies even in resistant tumors.

551

Bcl-2 overexpression inhibits TRAIL-induced apoptosis in type II cells

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Despite aggressive therapies, resistance of many tumors to current treatment protocols remains a major concern in clinical oncology and may be caused by defects in apoptosis programs. Since recent data suggest that TRAIL can bypass apoptosis resistance imposed by high Bcl-2 expression, we further investigated the role of Bcl-2 in TRAIL-induced apoptosis. In CD95-induced apoptosis, we previously identified two apoptosis signaling cell types. In type I cells, activation of effector caspases procedes independent of mitochondrial perturbations, whereas in type II cells, cleavage of effector caspases is mediated by mitochondria in a Bcl-2 dependent manner. Here we report that overexpression of Bcl-2 conferred protection against TRAIL-induced apoptosis only in type II cells such as neuroblastoma, malignant glioma or breast carcinoma cells indicating a cell type dependent (type I and type II) regulation of the TRAIL signaling pathway. In type II cells, Bcl-2 overexpression reduced TRAIL-induced cleavage of caspase-8 and Bid indicating that caspase-8 was activated upstream and also downstream of mitochondria in a feedback amplification loop. Importantly, Bcl-2 completely blocked cleavage of caspase-9, -7 and –3 into active subunits and cleavage of the caspase substrates DFF45 or PARP. Also, Bcl-2 blocked cleavage of the inhibitor of apoptosis protein XIAP upon TRAIL treatment. Likewise, overexpression of XIAP conferred resistance against TRAIL indicating that apoptosis was also amplified through a feedforward loop between caspases and XIAP. In contrast, in type I SKW lymphoblastoid cells, TRAIL-induced activation of caspase-8 directly translated into full activation of caspases, cleavage of XIAP, DFF45 or PARP and apoptosis independent of Bcl-2 overexpression. Interestingly, Bcl-2 similarly inhibited loss of mitochondrial membrane potential and the release of cytochrome c, AIF and Smac from mitochondria in both cell types. By indicating a cell type dependent regulation of the TRAIL signaling pathway, these findings may have important clinical implication. Thus, strategies targeting the molecular basis of resistance towards TRAIL, e.g. overexpression of Bcl-2, may be necessary in certain tumors for cancer therapy with TRAIL.

552

Overexpression of the G1 domain of PG-M/Versican induces overgrowth of human leiomyosarcoma cells by altering their proliferation-apoptosis equilibrium

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It has recently emerged that selected domains of the large ECM proteoglycan PG-M/versican may be involved in the control of different cellular processes. We have specifically investigated the role of the G1 hyaluronan-binding domain in the context of growth and dissemination *in vitro* and *in vivo* of malignant soft tissue sarcoma cells. SK-LMS-1 human leyomiosarcoma cells were stably transfected with a vector encompassing the G1 domain flanked by its signal peptide and the GFP reporter gene. G1 transfectants exhibited a significantly higher proliferation rate than the parental and mock transfectant cells. Cell proliferation was dose-dependently suppressed by soluble high Mr hyaluronan and hyaluronan oligosaccharides and this effect seemed to involve cell surface CD44. Overexpression of G1 domain did not affect adhesion or haptotatic migration of the sarcoma cells in a HA-free environment, whereas it significantly increased their invasive capabilities and caused a perturbed cell locomotion in the presence of HA. Transfectans also showed a higher cabapility of anchorage-independent growth which was associated with a markedly reduced apoptotic rate. In fact, cells were resistant to both cytotoxic drug-induced and Fas-dependent apoptosis. When inoculated subcutaneously into nude mice, G1 overexpressing cells gave rice to local, initially slowly growing tumour masses, which subsequently expanded significantly faster and reached larger sizes than those generated by wild-type and mock transfected cells, starting from the 4th week post-inoculation. Taken together these findings suggest that the over-secreted versican G1 domain may control the proliferation-survival-apoptosis equilibrium in leiomyosarcoma cells, through a putative pericellular HA sequestering. *In vivo* soft tissues sarcomas overexpressing PG-M/Versican may obey a temporary growth control exerted by HA, which is eventually de-repressed by their intrinsic apoptotic resistance.