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Letter to the Editor

SHP-1 sensitizes MCF-7 cells to trichostatin A-induced apoptosis by modulating PI3K-dependent events

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Dear Editor,

Of the several signaling molecules important for cell survival, one that has garnered much attention is the serine/threonine kinase Akt.[1] Downstream of phosphoinositide 3′-kinase (PI3K), Akt is activated by phosphorylation and regulates proapoptotic substrates, including Bad, caspase-9 and the forkhead transcription factor, which are all inactivated by phosphorylation.[2–4] We have previously demonstrated that the p85 regulatory subunit of PI3K interacts with, and is negatively modulated by, the cytosolic protein tyrosine phosphatase SHP-1.[5] SHP-1-mediated events are increasingly associated with genotoxic stress and, at least in our examination of somatostatin-induced apoptosis, appear to be dependent on caspase-8 recruitment, and increased p53 and Bax expression.[6,7] Activation of caspases during apoptosis is a process tightly regulated by various Bcl-2 family members.

The histone deacetylase (HDAC) inhibitors butyrate and trichostatin A (TSA) are gaining interest as potential anticancer drugs. Experimentally, TSA induces caspase activity and apoptosis in the MCF-7 breast cancer cell line *via* a cytochrome *c*-dependent pathway,[8] thereby implicating caspase-9-dependent events. Interestingly, both butyrate and TSA can induce a shift in cellular tyrosine phosphorylation; one mechanism involves repression of mRNA and protein expression of the c-*Src* tyrosine kinase by inhibiting activity of each of its promoters,[9] whereas the other mechanism involves induction of SHP-1 expression by increasing the activity of the tissue-specific P1 promoter in MCF-7 cells.[10]

We now demonstrate that the sensitivity of MCF-7 cells (HTB-22: ATCC) to TSA-induced apoptosis was heightened by stable overexpression of SHP-1, but not by overexpression of the catalytically inactive mutant of SHP-1, that is, SHP-1-C455S. Expression of p85, the regulatory subunit of PI3K, in the MCF-7 parental cell line was transiently increased by TSA treatment indicating activation of cell survival mechanism(s). In contrast, this transient increase was absent in SHP-1-overexpressing cells and p85 immunodetection was, in fact, completely lost by TSA-treatment for 48 h. Stable overexpression of SHP-1 during TSA treatment may induce an actual downregulation of total cellular p85 protein rather than a diminished phosphorylation of p85, which was proposed to account for the regulation of p85 activity by transient SHP-1 expression.[5,11] The reduction in phosphorylation of Akt observed in TSA-treated SHP-1 transfectants was not observed in SHP-1-C455S transfectants, concurring with the observation that SHP-1, but not the catalytic inactive mutant, can negatively regulate p56^{Lck}-induced phospho-PI3K activity and phosphorylation of Akt (Figure 1).[11]

Treatment with the tyrosine phosphatase inhibitor vanadate inhibits apoptosis by activation of PI3K/Akt, and phosphorylation of Bad.[12,13] The present study revealed that basal phosphorylation of Bad-Ser136 was decreased by SHP-1 overexpression and further affected by TSA treatment. The basal and response levels of phospho-Akt were similarly affected in these same cells and, thus, support the notion that

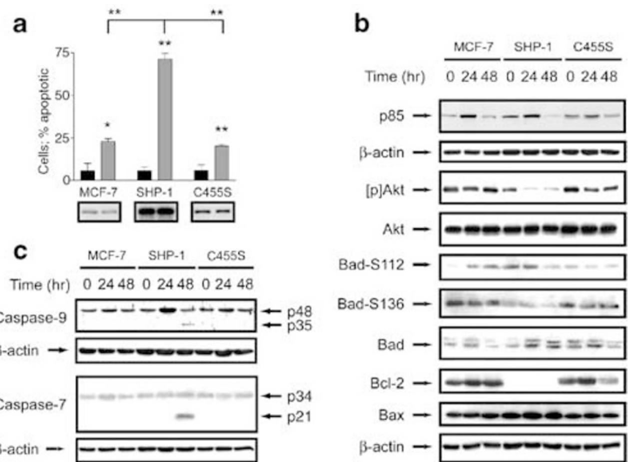

Figure 1 Effect of overexpressed SHP-1 on TSA-induced apoptosis in MCF-7 cell lines. (a) Neomycin-resistant pools of stable transfectants expressing wildtype SHP-1 [SHP-1] and the catalytic mutant SHP-1-C455S [C455S] were generated (the respective immunoblots are shown below the corresponding labels) and treated with TSA (300 ng/ml). Apoptotic events were assessed by fluorescence-activated cell sorting (using annexin-V-FLUOS; 10 000 gated events/sample). MCF-7-SHP-1 transfectants were extremely sensitive to TSA treatment. The results (mean $\pm$ S.D., $n \geq 3$) are presented as the percentage of apoptotic cells in control cultures (black bars) *vs* TSA-treated (48 h) cultures (gray bars). *: $P < 0.05$ and **: $P < 0.01$ (by ANOVA) *vs* the respective controls or between the indicated sampling groups. (b) TSA treatment induced a transient increase in expression of p85 in MCF-7 and a return to basal levels by 48 h. Expression of p85 in similarly treated MCF-7-SHP-1 cells was almost completely abolished by 48 h of TSA treatment. Phosphorylation of Akt ([p]-Akt) was selectively decreased by TSA treatment in MCF-7-SHP-1 cells [SHP-1]. TSA also induced proapoptotic changes in the phosphorylation and/or expression of Bcl-2 family members, for example, Bad, Bcl-2 and Bax, almost exclusively in the MCF-7 SHP-1 overexpressing cell line. (c) Cleavage of procaspase-9 to the active p35 subunit was detected only in MCF-7-SHP-1 [SHP-1] cells at 48 h of TSA treatment. The MCF-7 cell line is caspase-3 null, but does express the executioner caspase-7, which, because of substrate recognition, is able to cleave the same substrates as caspase-3. A strong 21 kDa band corresponding to activated caspase-7 cleaved from the 34 kDa precursor was only detectable in MCF-7-SHP-1 transfectants treated with TSA for 48 h. Cleavage fragment sizes are indicated on the right. Representative blots probed for β-actin are included in (b) and (c) to confirm equivalent protein loading.

phosphorylation of Bad-Ser136 is dependent on Akt kinase activation.[14] Basal phosphorylation of Bad-Ser112 was increased by SHP-1 overexpression, perhaps indicating a heightened cell survival response mechanism(s) to stress, and was diminished by TSA treatment of these cells. In contrast, increased phosphorylation of Bad-Ser112 was evident in TSA-treated MCF-7 parental cells, even though the phosphorylation of ERK1/2 in these same cells was greatly inhibited (data not shown). These combined data suggest either that phosphorylation of Bad-Ser112 is not as specific to ERK1/2 activation in MCF-7 cells as it has been demonstrated for other cell lines[14] or simply that a redundancy in cell survival signaling pathways is revealed when ERK1/2 activation is compromised. What is germane to the present hypothesis is that SHP-1 overexpression leads to a loss of Bad phosphorylation (at Ser112 and Ser136) by 48 h of TSA treatment. It is well known that unphosphorylated Bad sequesters antiapoptotic molecules such as Bcl-2 away from the proapoptotic Bax and, once liberated, Bax translocates to the mitochondrial membrane where it modulates the formation of the permeability transition pore. This event allows intramembranal cytochrome *c* to escape and to activate downstream caspases. The present observation of a complete ablation of Bcl-2 protein by SHP-1 overexpression concurrently with an increased expression of Bax protein would shift the cells' response from one promoting survival to one of a greater predisposition to an apoptotic outcome. This corroborates the angiotensin II-mediated (apoptotic) ERK inactivation, and the subsequent Bcl-2 inactivation and Bax upregulation observed in PC12W and R3T3 cells.[15] Our observation of proapoptotic changes in Bcl-2-related proteins coincides with increased sensitivity of MCF-7 cells to apoptogenic insult and the cleavage of caspase-9 and of the executioner caspase-7. Processing of these caspases corresponded to a 170% increase in the activity of caspase-9 [$F_{(3,15)}=3.775$, $P=0.0406$] and a 160% increase in the activity of caspase-7 [$F_{(3,15)}=6.592$, $P=0.0070$] (data not shown).

Perhaps what dictates sensitivity to apoptogenic stimuli is the level of expression of molecules such as Akt family members. It is known that AKT1/RACα is overexpressed in less aggressive human breast cancer cell lines such as MCF-7,[16] whereas AKT3/RACγ is overexpressed in more aggressive forms of breast tumors.[17] Our present data suggest that targeted manipulation of the Akt-dependent antiapoptotic signaling pathway in combination with a relative shift in cellular protein tyrosine phosphatase activity may provide a novel approach to enhancing the efficacy of adjuvant therapies in the clinic.

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