Caspase-9 is an initiator caspase that mediates apoptosis induced by cytotoxic agents. Cardone et al. (1) reported that Akt, a kinase that suppresses apoptosis, phosphorylates caspase-9, thereby preventing activation of this protease. This finding is of fundamental importance because it explains how growth factors can prevent cell death and because it demonstrates that caspases, a central part of the apoptotic machinery, can be regulated by phosphorylation.

Given that importance, we tried to establish a reason for our failure to detect phosphorylated caspase-9 in cell lines we have analyzed. In particular, we decided to test, using a functional assay, whether caspase-9 is regulated by Akt phosphorylation. We planned to reintroduce caspase-9 or its Akt phosphorylation site mutants into cells derived from mice deficient in caspase-9. We reasoned that the lack of endogenous caspase-9 would allow unambiguous detection of the mutations' effect.

The experiment remained unfinished, however, because we found that mouse caspase-9 had no Akt phosphorylation sites, despite the close similarity between human and mouse proteins (Fig. 1). Subsequently, we found that Akt phosphorylation sites were also absent in caspase-9 obtained from MDCK cells, a dog kidney cell line used by Cardone et al. (1) to make the original observation. Hence, Akt can phosphorylate caspase-9 in neither mice nor dogs. Such a variability among species runs counter to the observation that the Akt phosphorylation site in Bad—a protein that is involved in apoptosis and is regulated by Akt—is conserved from zebrafish to humans. Either it must be assumed that Akt regulates apoptosis differently in mice and humans, or the notion that caspase-9 is regulated by Akt phosphorylation has to be reexamined.

Response: We thank Rodriguez et al. for noting the difference in the predicted amino acid sequences of pro-caspase-9 between humans and other mammalian species, which implies that Akt does not regulate this cell death protease in mice, rats, and dogs. Fujita et al. (1) also observed this sequence difference and demonstrated that Akt does not phosphorylate mouse caspase-9 in vitro. The observation that shorter-lived lower mammals lack the Akt phosphorylation site in caspase-9 suggests that this phosphorylation site evolved relatively recently in humans, providing an additional way of regulating the cytochrome c pathway that couples mitochondrial damage to caspase activation.

Though the absence of consensus Akt phosphorylation sequences in caspase-9 of shorter-lived mammals is interesting, it does not detract from the significance of our findings (2). Our work provided the first example of protease regulation by phosphorylation, which reveals a novel paradigm for modulating the activity of proteases in cells. Defects in the pathways regulating Akt have been documented in a large proportion of human cancers; our data showed that in human tumor cell lines, Akt-mediated phosphorylation of caspase-9 represents one mechanism used by Akt to suppress apoptosis. Thus, although the findings of Rodriguez et al. argue against a role for caspase-9 phosphorylation in normal, evolutionarily conserved processes (such as fetal development) in which programmed cell death is important, they do not exclude a role in humans for either too much or too little Akt-mediated phosphorylation of caspase-9 in pathologies (such as cancer or neurodegenerative diseases) where cell accumulation or cell death occurs.

All analysis of caspase-9 phosphorylation was performed in human cell lines, rather than rodent or canine cells, and we stand by those data (2). Also, in the experiments involving cytosolic extracts from murine and canine cell lines transfected with active Akt or Ki-Ras (which is known to cause Akt activation), we added in vitro translated human pro-caspase-9 and monitored cytochrome c-induced processing and activation of human pro-caspase-9 in vitro. Our published data, however, also revealed a suppression of cytochrome c–inducible effector caspase activity in cytosolic extracts prepared from murine and canine cells with elevated Akt activity (2). These data, together with the apparent absence of consensus Akt phosphorylation sites for mouse and dog caspase-9, imply the existence of an additional, evolutionary conserved mechanism by which Akt can suppress activation of caspases in the cytochrome c (mitochondrial) pathway for apoptosis. Candidates for this target of Akt regulation might include caspase-9 activators, particularly Apaf-1 (3); caspase-9 suppressors such as XIAP (4); unidentified proteins that modulate the cytochrome c/Apaf-1 apoptosome; and downstream caspases such as caspase-7, which contains a candidate Akt phosphorylation site (RxRXXT) that is conserved in both mice and humans.

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26 August 1999; accepted 3 February 2000

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20 December 1999; accepted 3 February 2000