Caspase-8: Fly or Die

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Abstract

Recent studies have revealed that procaspase-8 has an important function in cell adhesion and motility. Src phosphorylation controls this function by preventing the conversion of procaspase-8, which is an adhesion/migration factor, to mature caspase-8, which is an apoptosis-inducing factor. This provides a mechanism to switch these opposing functions. In its migratory role, procaspase-8 interacts with the phosphatidylinositol-3-OH kinase regulatory subunit p85α and c-src to modulate signaling by Rac and extracellular signal-regulated kinase, and promote calpain activation. Here, I survey the findings of these studies and discuss potential mechanisms and ramifications for cancer prognosis and therapy. [Cancer Res 2008;68(12):4491–3]

A Cell Migration/Adhesion Function for Caspase-8

Caspase-8 is well-characterized as an initiator of death receptor-mediated apoptosis and has been implicated in other apoptotic responses as well. As with other caspases, it has long been intuited that caspase-8 may not have evolved solely to kill cells (1). In particular, the decision of a cell to undergo apoptosis versus cell migration might, in principle, be controlled by a molecular toggle, due to the mutual exclusivity of these phenomena, i.e., migration involves transient cytoskeletal changes that might otherwise engage an anoikis response, and, conversely, suppressing the migration of apoptotic cells may prove physiologically useful.

Several recent observations have provided clues that caspase-8 may possess a “moonlighting function” related to this molecular toggle, albeit hypothetical. Caspase-8 knockout mice die during early embryogenesis, and tissue-specific knockouts have developmental defects that cannot be explained by deficits in apoptosis (2). Transcription through the nuclear factor-κB pathway and proliferation of T cells is also downstream of caspase-8 (3). Moreover, although caspase-8 expression is compromised in a restricted subset of human tumors, it is maintained or up-regulated in many common tumor types, consistent with a nonapoptotic, possibly prometastatic function (4, 5).

Stimulated by these cues, caspase-8 was found to promote cell migration and cell-matrix adhesion (6). These findings were confirmed and extended by additional studies (5, 7, 8), which defined a new function for caspase-8 that could explain its developmental effects as well as the observation that activated FAS promotes invasion (9). Thus, incorporating the new findings, the function of caspase-8 can be conceptualized as a unique “fly or die” switch. Here, I summarize and critically evaluate the mechanisms that have been proposed by the recent studies (5–8).

Caspase-8 Functions Upstream of Calpain-2

Initial findings documented the cell motility/migration and cell-matrix adhesion effects of caspase-8 in knockout mouse embryo fibroblasts, the caspase-8–null neuroblastoma cell line NB7, and other cell lines in which caspase-8 was inactivated by crmA protein (6). Interestingly, although cell motility and adhesion were found to be stimulated by caspase-8 in these cell lines, a paradoxical effect—i.e., inhibition of cell adhesion in normal (i.e., nontransformed) epithelial cell lines—was noted, indicating that the effect of caspase-8 may differ in epithelial versus fibroblastic or tumor cells.

A potential role for caspase-8 in regulating calpain activation was indicated (6). Calpain activation by the activated epidermal growth factor (EGF) receptor is important in most or all aspects of cell migration: lamellipodial extension, rac activation, trailing edge detachment, and focal adhesion turnover, as well as other aspects of cell behavior such as cell-matrix adhesion and high fidelity of cytokinesis, that is, suppression of multinucleate cell formation (10, 11). The caspase-8-dependence of calpain activation was indeed reflected in an effect of caspase-8 on those four of the six calpain-dependent processes listed above that were tested; the aberrant protrusions found in spreading caspase-8–null cells remarkably resembled those of calpain-2–null cells (12).

How could caspase-8 regulate calpains? Calpain-2, an important isof orm for cell migration, is regulated in part by extracellular signal-regulated kinase (ERK)-mediated phosphorylation on serine-50 (13). In this connection, a contribution of caspase-8 to EGF-stimulated ERK activation was observed (5, 8). This phenomenon—which is consistent with the stimulation of ERK by TNFR1 and FAS (14, 15)—suggests one potential mechanism for caspase-8 to regulate calpain-2 as well as other migration/adhesion pathways that are ERK dependent. It is not yet clear how caspase-8 regulates ERK activation. One report attributed this regulation, surprisingly, to the Death Effector Domains (DED; i.e., prodomain) of caspase-8 (8). Consistent with this idea, a direct association between ERK and a DED-containing fragment of caspase-8, and co-transport of an ERK-caspase-8-DED complex to the nucleus during apoptosis has been reported (16). Although a corresponding interaction between the DEDs of (full length) caspase-8 and ERK in nonapoptotic cells is hypothetically possible, we have neither observed this interaction nor evidence of caspase-8 fragmentation in nonapoptotic cells.1 An alternative possibility is that the DEDs localize caspase-8 such that it activates kinases upstream of ERK, using either a binding/conformational switch mechanism (favored in one cell system where the catalytic activity was dispensable; refs. 5, 8) or through the proteolytic activation of kinases by caspase-8 (in other cell contexts) or calpains.

1 S.M. Frisch, unpublished data.
Interactions of Caspase-8 with p85<sub>a</sub> and c-src: Mechanisms and Ramifications

A second, nonmutually exclusive mechanism for cell migration/adhesion effects of caspase-8 involving novel caspase-8 protein interactions was posited. Caspase-8 exists as a ~55 kDa precursor, procaspase-8, which is activated by FADD or c-FLIP–mediated dimerization (17). During an apoptotic response, dimeric caspase-8 cleaves itself at three sites, first at D374 and D384, producing an enzymatically active, DISC-bound species, then at D216, releasing the mature caspase-8 tetramer into the cytoplasm (Fig. 1). An uncleavable mutant form of caspase-8 (D374A/D384A) that constitutively remains in the procaspase-8 form—the form that is mainly or solely detected in nonapoptotic cells—cannot support FASL-induced apoptosis (18). Might procaspase-8 perform the nonapoptotic functions of caspase-8?

An uncleavable mutant of caspase-8 restored adhesion/motility as efficiently as wild-type caspase-8 (5) and these activities did not require the active-site cysteine (5, 8). These observations reveal the first biological functions of procaspase-8, other than serving as a precursor for the mature enzyme. How procaspase-8 might achieve this activity was postulated as follows.

Recently, c-src was reported to phosphorylate caspase-8 at tyrosine-380 (Fig. 1), blocking caspase-8 autocleavage at D374/D384 and suppressing FASL-induced apoptosis (19). The activity of c-src is frequently elevated in cancer cells, and is activated transiently by motility factors such as EGF (20); consistent with this, increased caspase-8 tyrosine-380 phosphorylation was detected in cancer cells with hyperactive src (19). These observations provide a novel mechanism by which c-src protects cells against apoptosis, by maintaining caspase-8 in the proform.

Interestingly, the phosphorylation acceptor site, tyrosine-380, was required for cell migration and adhesion, elevating procaspase-8 from a mere protease precursor to a phosphorylation-regulated signaling switch for fly or die (5, 7).

These groups then pursued the function of tyrosine-380, generating different but nonmutually exclusive results. The first group showed that phosphorylated caspase-8 interacts with the p85<sub>a</sub> regulatory subunit of phosphatidylinositol-3-kinase, requiring tyrosine-380 and phosphorylation by c-src or c-fyn (5). They hypothesized that this interaction might affect Rac activation through a direct or indirect mechanism, perhaps involving the p85-Rac complex reported earlier (21). The second group showed that c-src is in a complex with phosphorylated (but not unphosphorylated mutant) caspase-8, and postulated that this complex is important for cell motility (7). This is plausible in that it is not unusual for src substrates to complex stably with c-src (20). Phosphorylated caspase-8—but not a Y380F mutant—localized to

Figure 1. Model: Src phosphorylation switches caspase-8 function. Under apoptotic stimulation, procaspase-8 undergoes autocatalytic cleavage to generate the proapoptotic mature tetramer. However, upon stimulation with motility factors such as EGF, tyrosine kinases including c-src phosphorylate caspase-8, preventing its autocatalysis and enabling an interaction with p85<sub>a</sub>. This interaction, as well as potential (direct or indirect) interactions with c-src (dotted lines), stimulates cell migration and adhesion through molecules including Rac, calpain-2, and ERK.
membrane ruffles associated with early cell attachment. Interestingly, src interacts directly with p85α (through an SH3-polyproline binding event), raising the possibility of either caspase-8-p85-src and/or caspase-8-src-p85 ternary complexes (22). In this connection, a c-yes-mediated recruitment of p85α to CD95 that stimulates globstoma invasion has been reported recently (23).

The third group reported no role for tyrosine-380 in cell adhesion (its role in cell migration was not addressed) and, as mentioned above, attributed cell adhesion, ERK activation, and src interaction to the DEDs (8). Although seemingly contradictory, these negative results (with regard to the cell adhesion effect of tyrosine-380) arose from a conventional adhesion assay, which presumably reflects integrin expression and affinity, whereas the positive data arose from Electrical Cell-Substratum Impedance Sensing, which measures adhesion and cell spreading. Thus, these observations are not incompatible, in that different end points were measured, although the same term was used to describe them. The attribution of caspase-8-src interaction and ERK activation to the DEDs in one study (8), but tyrosine-380 in the other (7) might well reflect the contributions of both elements.

Summary and Future Perspectives
In summary, the four recent papers on cell migration/adhesion of procaspase-8 indicate that the processing of caspase-8, which is controlled in part by tyrosine phosphorylation, is an important migration/adhesion versus apoptosis switch mechanism. Elucidation of the regulatory mechanisms involved will undoubtedly yield new connections and new surprises. From a clinical point of view, it may prove useful to characterize the expression and phosphorylation state of caspase-8 in tumors, to assess the feasibility of using this protein as a prognostic marker or to pharmacologically stimulate caspase-8 processing.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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