CELL DEATH

# Death by self-digestion

Think of cell death and you probably think of caspase-executed apoptosis. Cells going through tough times can also undergo self-digestion — autophagy — but only now have Li *et al.* discovered that this directly causes cell death and found some clues to how it does so.

Several factors cause mouse fibroblasts to die independently of caspase activity. Rather unexpectedly, though, a broad-spectrum caspase inhibitor zVAD also induced death in these, and other, cells. Cellular post-mortem examination uncovered death traits — in particular, membrane-bound vacuoles — that were different from those seen in apoptosis and, instead, resembled autophagic traits. Could autophagy condemn cells to die?

Although two inhibitors of autophagy prevented zVAD-induced death, these compounds inhibit phosphatidylinositol 3-kinase, and therefore might kill cells by other means. So Li *et al.* directly assessed the effect on cell death of inhibiting genes that are required for autophagy (*ATG* genes) using RNA inhibition (RNAi). Reduced expression of *ATG7* virtually inhibited zVAD-induced death, as did reducing the levels of another ATG gene, *beclin-1*. In both cases, the lack of cell death correlated with an inhibition of autophagic vacuole formation, so ATG7 and beclin-1 are necessary for zVAD-induced non-apoptotic cell death.

Although the mechanisms of autophagy aren't well understood, non-apoptotic death can arise from death receptors through the serine/threonine kinase RIP (receptor-interacting protein). Inhibiting RIP by RNAi decreased both autophagy and death. RIP can activate JUN amino-terminal kinase (JNK), and JNK was activated in response to zVAD treatment. RNAi treatment of JNK or its upstream kinase MKK7 (mitogen-activated protein kinase kinase-7) blocked zVAD-induced autophagy and death. Inhibiting protein synthesis in zVAD-treated cells also blocked cell death, implying that new protein (or proteins) needs to be made, possibly through the transcription of target genes. One transcription factor that lies downstream of JNK is JUN, which, when inhibited, blocked autophagy and cell death by 45–50%.

So a RIP–MKK7–JNK–JUN pathway is involved in autophagy and cell death, but how does zVAD induce this? Because caspase-8 is involved in lymphocyte-receptor signalling in non-cell-death situations, Li *et al.* studied its role in autophagy. Depleting caspase-8, but not caspases-1, -2, -3, -9 or -12, using RNAi increased cell death with autophagic traits, and, because caspase-8 is potently inhibited by zVAD, it seems likely that zVAD induces cell death by inhibiting caspase-8.

So this caspase-8-mediated suppression of autophagic death points to caspases being regulators of apoptotic and non-apoptotic cell death — which has important implications for the development of caspase inhibitors for therapeutic treatment.

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**References and links**

**ORIGINAL RESEARCH PAPER** Yu, L. *et al.* Regulation of an *ATG7–beclin 1* program of autophagic cell death by caspase-8. *Science* 6 May 2004 (doi:10.1126/science.1096645)

CANCER

# Proteins perish under spotlight

Genes hold vital information, but ultimately, proteins have the final say. So not only do proteomic-based screens that knockdown surface proteins, described by Eustace *et al.* in *Nature Cell Biology*, have advantages over current high-throughput genomic approaches, but, in this case, they've also unearthed an unexpected candidate, heat-shock protein 90α (Hsp90α), in the quest to identify proteins that function in tumour invasion.

The screens used either monoclonal antibodies or antibodies generated by phage display to target light-induced damage to surface proteins on invasive HT-1080 cells. In both cases, fluorophore-assisted light inactivation (FALI) using fluorescein-labelled binders damaged the bound proteins *in situ*. The FALI-treated cells were then assayed for their ability to invade a membrane that was coated in Matrigel (extracellular matrix that is secreted by sarcoma cells).

In the cases in which invasion was inhibited, the cognate antigens (that is, the surface proteins recognized by the antibodies) were identified using immunoprecipitation and mass spectrometry. Reassuringly, proteins such as $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins, which are known to function in adhesion, migration and invasion, were uncovered.

Surprisingly, though, both screens identified the molecular chaperone Hsp90α. The authors first confirmed, by various means, the location of Hsp90α, as there was little prior evidence of it being an extracellular protein. Hsp90α surface expression was not restricted to HT-1080 cells — another invasive cell line, MDA-MB231, also expressed it. It was also found in conditioned medium, indicating that it had been secreted.

Pharmacological inhibitors of Hsp90α, or FALI using Hsp90 antibodies, inhibited the invasion of MDA-MB231 cells. So how does extracellular Hsp90α mediate such invasion? Could it be functioning similarly to its intracellular counterpart by promoting the proper activity and function of its substrates?

Eustace *et al.* reasoned — correctly, it transpired — that Hsp90α might help to activate matrix metalloproteinase-2 (MMP2), a matrix-degrading enzyme that is expressed at high levels in invasive tumours. Hsp90α could immunoprecipitate MMP2 from conditioned medium, so the two proteins directly interact. Importantly, specifically inhibiting extracellular Hsp90 (using an Hsp90 inhibitor attached to beads) decreased MMP2 activity, and the consequent decrease in invasion was overcome by adding back active MMP2.

Hsp90α might therefore increase metastasis by activating MMP2 and increasing the enzymatic digestion of the environment surrounding tumours. Clinical trials using general Hsp90 inhibitors are already underway, but inhibiting extracellular Hsp90α might provide a more targeted approach.

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**References and links**

**ORIGINAL RESEARCH PAPER** Eustace, B. K. *et al.* Functional proteomic screens reveal an essential extracellular role for Hsp90α in cancer cell invasiveness. *Nature Cell Biol.* **6**, 507–514 (2004)