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Differentiation is death

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Summary

The apoptosis signaling molecule caspase 3 is also required for differentiation in myoblasts

Significance and context

In myogenesis, muscle stem cells exit the cell cycle and fuse to form myotubes, the precursors of muscle cells. Myoblasts that fail to exit the cell cycle following commitment to this fate undergo apoptosis and die. In the apoptotic cascade, various caspases are activated by proteolytic cleavage, and the activity of one of these, caspase 3, has always been considered to be a specific marker of apoptosis. The report by Fernando *et al*. that this molecular marker of apoptosis now seems to be a necessary prerequisite of muscle-cell differentiation thus shatters the paradigm for those studying apoptosis during myogenesis.

Several lines of evidence led Fernando *et al.* to investigate whether apoptosis and myoblast differentiation might initially share a common pathway. At the molecular level, caspase 3 has been linked with activation of the mitogen-activated protein (MAP) kinases (MAPKs), Jun N-terminal kinase (JNK) and p38, which are involved in the initiation and continuation of myogenesis. Certain cellular events, namely cell-membrane blebbing and fusion and actin fiber reorganization, appear to be common to both myogenesis and apoptosis. Finally, caspase-3-knockout mice appear to be quite underweight and have visibly less muscle mass than heterozygous individuals.

Key results

In wild-type cell lines, raised levels of caspase 3 were detected after initiation of differentiation (which is achieved by placing actively growing cells in low-serum medium). Immunocytochemistry was used to show that the observed increase in caspase 3 was associated with differentiating myoblasts and not apoptotic cells. After initiation of differentiation in cell lines derived from caspase-3-knockout mice, there was a measurable lack of myotube formation compared with cell lines derived from heterozygous and wild-type mice, even though cell proliferation was comparable before the initiation of differentiation. Lower levels of differentiation-specific gene products, such as myogenin and hypophosphorylated MyoD, were found in these cells, while levels of cyclin D1, a marker for cellular proliferation, were higher. To rule out the possibility that apoptosis was somehow removing inhibitory

cells, or that it could have some kind of cell-autonomous effect leading to the triggering of myogenesis, apoptosis was measured in both normal and knockout cell lines. Surprisingly, no difference in the degree of apoptosis was found between the two, and, therefore, the raised levels of caspase 3 in the normal cell lines did not appear to be involved entirely with apoptosis.

To complement these experiments, caspase 3 was chemically inhibited in normal cell lines. The lack of myotube formation in these cells was very similar to that observed in the caspase-3-null cell line, and the expression of the late myogenic marker MEF2C (myocyte enhancer binding factor-2) was almost absent from the inhibited cell lines. Additionally, activated caspase 3, transfected as a construct into subconfluent normal myoblasts that were proliferating in full medium, caused these cells to begin to undergo differentiation, and these cells ceased to express cyclin D1. The authors then demonstrated the possible involvement of a downstream effector of caspase 3, namely MST1 (mammalian sterile twenty-like kinase), by transfecting the activated MST1 transcript into caspase-null cell lines, which restores the ability of these cells to differentiate.

Conclusions

All the results indicate that endogenous caspase 3 activity is required for the differentiation of myoblasts into myotubes, and that MST1, activated by caspase 3, is in this pathway. It is therefore reasonable to conclude that the signaling pathways that lead to apoptosis and myogenesis are identical, or overlap, at least to begin with. The authors point out that, as similar signaling proteins such as MST1, MKK6 and p38 are also present in many other differentiated cell types, it is not out of the question that a similar picture will emerge in other terminally differentiated cell lineages, such as neurons.

Reporter's comments

Cells committed to myogenic differentiation that fail to exit the cell cycle enter apoptosis, but the signaling cascade that leads to myogenesis appears, at the start, to be identical to that of apoptosis. It is possible, therefore, that all cells at the start of the myogenic differentiation pathway may actually be committed to apoptosis, and only by exiting the cell cycle do they escape death and continue down a different signaling pathway. Once a cell becomes committed to a developmental pathway, however, it is doomed ultimately to senescence and death. One way or another, then, differentiation is death for these cells.

Table of links

Proceedings of the National Academy of Sciences of the United States of America

References

1. Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA: Caspase 3 activity is required for skeletal muscle differentiation. Proc Natl Acad Sci USA. 2002, 99: 11025-11030.