

2.3 Detection of apoptosis-related proteins

There are a number of genes that regulate apoptosis. That is, the products of these genes interfere with apoptotic pathways. Assays to detect the proteins encoded by these genes can complement the assays described in the previous sections.

The study of apoptosis-regulating genes and gene products is still evolving. The picture so far is complex (as summarized in Section A 1.3 of this guide). For instance, in some cases, the same gene has an effect on both the survival of normal cells and the development of cancers by preventing apoptosis³⁶. A detailed discussion of the field is beyond the scope of this guide, but is covered in a number of reviews^{36, 37}. As an introduction to the field, we discuss the characteristics of a few of these apoptosis-regulating proteins.

The relationship of the *ced* (*caenorhabditis elegans* cell death) genes to apoptosis in the nematode *Caenorhabditis elegans* has been extensively studied³⁸. Of these, the *ced-3* and *ced-4* genes³⁹ encode proteins that must be active to initiate apoptosis. In contrast, the *ced-9* gene product protects cells from apoptotic cell death, ensuring their survival⁴⁰. In other words, apoptosis is more likely when levels of *ced-3* and *ced-4* protein are high or when levels of *ced-9* protein are low.

In mammalian systems, the *Bcl-2* protooncogene serves much the same function as *ced-9*, blocking the induction of cell death⁴¹. The *Bcl-2* oncoprotein also protects against the cytotoxic effects of certain drugs⁴².

The *Bcl-2* protein can dimerize with itself or with the product of the *bax* gene⁴³. The presence of the *bax* protein seems to counteract the anti-apoptotic activity of *Bcl-2*. In summary, apoptosis is more likely when *bax* protein levels are high or when *Bcl-2* protein levels are low.

Another mammalian gene product, *p53*, is a tumor suppressor because it induces apoptosis in potentially malignant cells⁴⁴. Absence or mutation of the *p53* gene product led to malignant transformation and immortalization of the cell.

Increases in expression of a growth stimulating gene, the *c-myc* proto-oncogene, actually induces apoptosis in the absence of other growth factors^{45, 46}. High levels of the *Bcl-2* protein can counteract the effect of the *c-myc* protein.

For the analysis of apoptosis-regulating proteins, Roche Applied Science offers an ELISA kit for the detection of *p53* in fluids or extracts and antibodies to detect *c-myc* and Fas (CD95/Apo-1).

Cell surface receptor genes (APO-1/Fas/CD95), other growth-stimulating genes (*e.g.*, Ras), and other tumor-suppressing genes (*e.g.*, Rb) have also been implicated in the regulation of apoptosis^{2, 37}. The Fas (CD95/APO-1) molecule has originally been identified as a cell surface receptor that could mediate apoptotic cell death of transformed cells and cause regression of experimental tumors growing in nude mice. The function of Fas was assessed by establishment of mouse cell transformants that constitutively expressed human Fas. When the transformed cells were treated with the antibody to human Fas, the cells died by apoptosis within 5 hours, which indicated that Fas can transduce an apoptotic signal and that anti-Fas works as an agonist. The subsequent purification of human APO-1 antigen and molecular cloning of its cDNA established the identity of APO-1 and Fas. Meanwhile, numerous reports have shown a pivotal role of Fas in various physiological and pathological processes. The Anti-Fas provided by Roche Applied Science is suitable for the induction of apoptosis as well as for the detection of the Fas receptor.