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Insulin-like Growth Factor I (IGF-I) and the IGF-I Receptor Prevent Etoposide-induced Apoptosis[1]

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ABSTRACT

The interaction of insulin-like growth factors (IGF) with the IGF-I receptor promotes cell proliferation and survival. We examined the role of the IGF-I receptor as a possible direct inhibitor of apoptosis induced by the topoisomerase I inhibitor etoposide. When exposed to this agent, BALB/c 3T3 cells that constitutively overexpress the human IGF-I receptor (p6 cells) arrested in S phase and subsequently underwent apoptosis as determined by the appearance of a pre-G_1 apoptotic peak when studied by flow cytometry and the characteristic internucleosomal fragmentation of DNA. The addition of IGF-I markedly inhibited etoposide-induced apoptosis in a concentration-dependent manner. IGF-I was not mitogenic in the presence of etoposide. IGF-I was less effective in preventing apoptosis in parental BALB/c 3T3 cells and had no effect on etoposide-induced cell killing of mouse embryo fibroblasts that have a targeted disruption of the IGF-I receptor gene. These results demonstrate an important role for the IGF-I receptor as an inhibitor of apoptosis, independent of its mitogenic actions.

INTRODUCTION

The activation of the IGF-I[3] receptor by its ligands IGF-I, IGF-II, and insulin plays a critical role in growth and development (1–3). The specific role of the IGF-I receptor in development has been recently revealed by DeChiara *et al.* (4) and Liu *et al.* (5), who showed that targeted disruption of the IGF-I receptor gene (as well as the ligands IGF-I and IGF-II) resulted in profound fetal growth retardation. Cells cultured from these embryos proliferate slowly in the presence of serum, and their growth in serum-free medium cannot be supported by the same purified growth factors that sustain the growth of wild-type cells (6, 7). Most cell types require IGF-I for growth in culture (2, 8). In addition to its role in cell cycle progression, IGF-I appears to play an important role in cell survival. Several cell types, including hematopoietic cells (9, 10) and neuroglial cells (11–15), require IGF-I for survival. IGF-I also protects neuroglial cells from several forms of direct injury, including ischemia (16–20).

The survival of cells both *in vivo* and in culture represents the balance of cell proliferation and death. Whereas many noxious influences may directly cause tissue and cell necrosis, the induction of programed cell death, often termed apoptosis, represents a major mechanism of cellular demise (reviewed in Ref. 21). Distinctive morphological features of apoptosis include nuclear and cytoplasmic condensation with the formation of membrane-bound apoptotic bodies containing cytoplasmic organelles and nuclear fragments. Apoptosis is also accompanied by characteristic internucleosomal chromatin degradation.

The specific mechanism by which IGF-I promotes cell survival has not been clarified. IGF-I may directly prevent apoptosis (17, 19, 22). However, IGF-I could also promote cell culture survival by inducing the proliferation of viable cells within a damaged cell population. To address this issue, we have studied the effect of IGF-I on apoptosis of mouse BALB/c3T3 cells induced by the topoisomerase I inhibitor etoposide. We demonstrate that, whereas IGF-I alone inhibits apoptosis following S phase arrest by etoposide, cell survival is considerably enhanced in cells that overexpress the IGF-I receptor.

MATERIALS AND METHODS

Cell Lines and Materials. BALB/c3T3 cells were obtained from the American Type Culture Collection. The p6 cell line was derived from BALB/c3T3 cells by transfection of the human IGF-I receptor expression plasmid CVN-IGF-I-R (23, 24) in which the IGF-I receptor cDNA is under the control of the early SV40 promoter and expresses approximately 5×10^5 IGF-I receptors/cell. The generation of mouse embryo fibroblasts that have targeted disruption of the IGF-I receptor (R^- cells) has been described elsewhere (6).

Human recombinant IGF-I was obtained from Gibco BRL (Gaithersburg, MD). Etoposide was purchased from Sigma (St. Louis, MO). All other chemicals and biochemicals were of the highest purity commercially available.

Cell Culture. All cells were passaged in Dulbecco's minimal essential medium containing 10% calf serum. For experiments with serum-free medium, 5×10^3 cells were seeded in 35-mm plates in the presence of serum-containing medium for 24 h prior to being placed in serum-free medium supplemented with 0.1% BSA (fraction V) and 1 μM $FeSO_4$ and the indicated concentrations of etoposide or IGF-I. At the times indicated, the plates were trypsinized and cell counts were determined by counting in a hemocytometer. Cell viability was determined by trypan blue exclusion.

DNA Fragmentation. Cytosolic DNA was isolated for fragmentation analysis by lysing 10^6 cells in buffer containing 50 mM Hepes (pH 7.4) and 0.5% Triton X-100. The nuclei were centrifuged, and the cytosolic fraction was treated with RNase A (50 units/ml) for 20 min. DNA was then extracted with phenol:chloroform, 28:1, precipitated by the addition of 0.1 volume of 3.3 M sodium acetate and 2 volumes of ethanol, and visualized by electrophoresis in 2% agarose.

In some experiments, cells were labeled with [^{14}C]thymidine (Amersham, Arlington Heights, IL; final concentration, 0.25 μCi/ml) for 16 h prior to incubation with etoposide. At the indicated times, fragmented low-molecular-weight DNA was isolated as described above, and its radioactivity was quantified by liquid scintillation counting. Radiolabeled low-molecular-weight DNA released into the medium was also quantitated. The percentage of fragmented DNA was determined by measuring the amount of radiolabel in the cytosolic fraction (cellular + released) as a percentage of total radiolabeled DNA (nuclear + cytosolic + released).

Flow Cytometry. For analysis of cells by flow cytometry, cells were washed with PBS and fixed by the addition of 70% ice-cold ethanol. After 10 min, cells were washed with PBS and treated with RNase A (75 units/ml) for 30 min at 37°C, washed again in PBS, and resuspended in PBS containing 15 μg/ml propidium iodide. A minimum of 20,000 cells were analyzed with a Coulter Epics Profile II (Coulter Electronics, Inc., Hialeah, FL).

Statistics. All data represent the means ± SE of triplicate determinations and are representative of at least three experiments. Statistical significance was determined by Student's *t* test.

RESULTS

The topoisomerase inhibitor etoposide prevents the religation of DNA by stabilizing the association of topoisomerase with the 5′ end of DNA during DNA replication (25) thereby inducing S phase arrest. Fig. 1 demonstrates the effect of IGF-I on p6 cell killing by etoposide.

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[3] The abbreviation used is: IGF, insulin-like growth factor.

In the presence of 2 μM etoposide, only about 50% of viable cells remained after 48 h (●). Higher concentrations of etoposide up to 40 μM only slightly further decreased cell viability. Addition of IGF-I to serum-free medium inhibited etoposide-induced cell death by >60%. At the highest concentration of etoposide tested (40 μM), IGF-I was less effective but still prevented loss of cells by approximately 50%. Fig. 2 illustrates the concentration dependence of the protective action of IGF-I. Significant inhibition of cell death was obtained at 50 ng/ml of IGF-I, and even greater protection was observed at 100 ng/ml of IGF-I, the highest concentration tested.

The preservation of cell viability by IGF-I in the presence of etoposide could be due to the direct prevention of cell death or, alternatively, could reflect enhanced cell proliferation of cells that are not lethally affected by etoposide. To assess these possibilities, cells were treated with etoposide (20 μM) in the presence or absence of IGF-I (50 ng/ml). After 24 h, all attached viable cells as well as both attached and detached dead cells were counted. In the presence of etoposide, the total number of cells was not increased by IGF-I (data not shown). Thus, etoposide effectively prevented p6 cell mitogenesis even in the presence of IGF-I, and IGF-I directly prevented cell death under these conditions. In the following experiments, we determined whether the prevention of etoposide-induced cell death by IGF-I is due to the prevention of apoptosis.

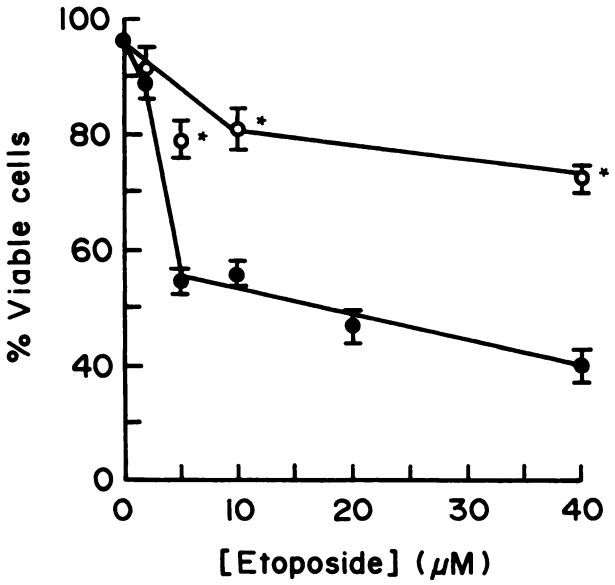


Fig. 1. Effect of IGF-I on etoposide-induced cell killing. p6 cells (5×10^3/plate) were incubated for 24 h in the presence of 10% FCS and then maintained for an additional 48 h in serum-free medium in the presence of the indicated concentrations of etoposide either with (○) or without (●) IGF-I (50 ng/ml). The plates were then gently washed, and the number of viable and dead cells was determined by counting in a hemocytometer. *Points* (*bars*) are the means (±SE) of triplicate determinations from a representative experiment. *, $P < 0.05$ compared to etoposide alone.

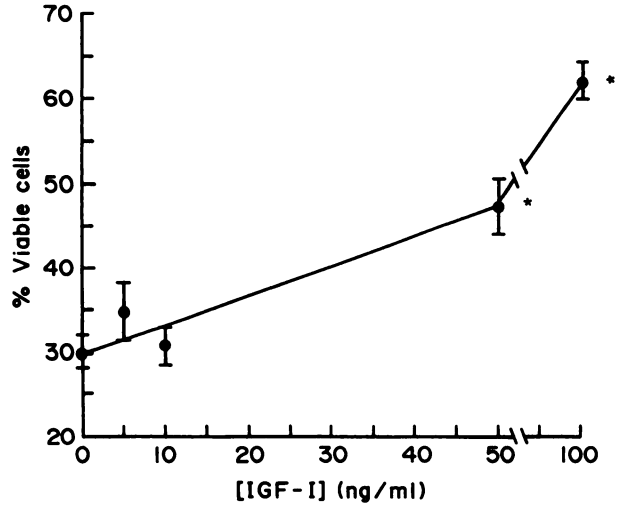


Fig. 2. Concentration dependence of IGF-I for protection from etoposide-induced p6 cell killing. p6 cells were treated with 20 μM etoposide, as described for Fig. 1, in the presence of the indicated concentrations of IGF-I. Cell counts were determined after 48 h. *Points* (*bars*) are the means (±SE) of triplicate determinations from a representative experiment and represent the percentages of viability in the absence of etoposide. *, $P < 0.05$ compared to etoposide alone.

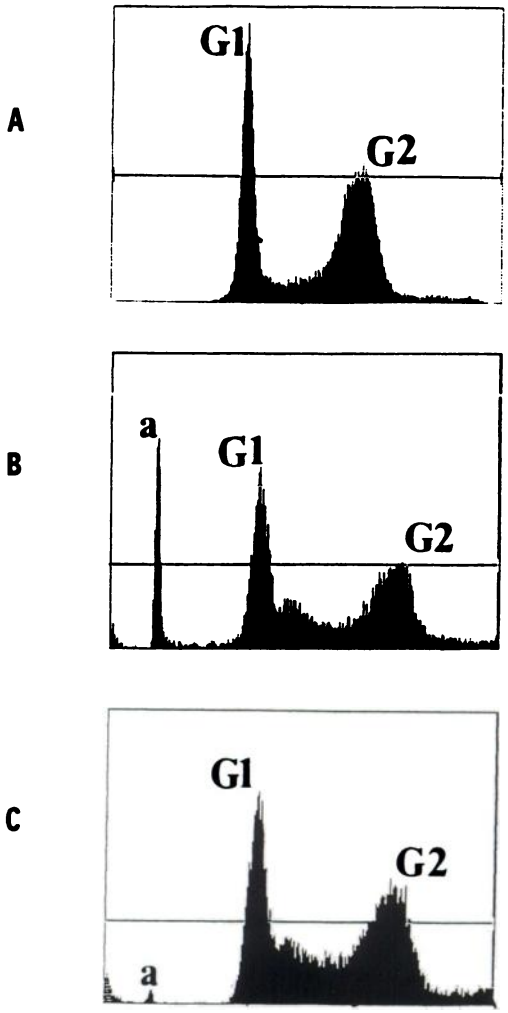


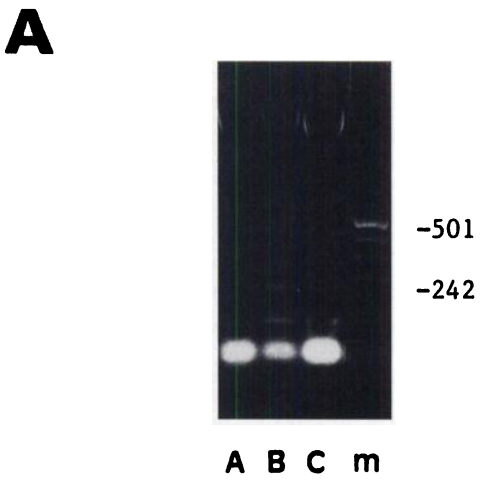
Fig. 3. Flow cytometry of p6 cells. P6 cells were treated with etoposide (20 μM) either with (*C*) or without (*B*) IGF-I (50 ng/ml). *A*, control cells without additions. Cells were collected after 6 h and processed for flow cytometry as described in "Materials and Methods."

Fig. 3 demonstrates the effect of etoposide on the cell cycle parameters as measured by flow cytometry. When cycling p6 cells were treated with etoposide, an increase in S phase cells appears within 4 h (Fig. 3*B*), as well as a distinct peak of pre-G_1 cells corresponding to an apoptotic cell population (26). The apoptotic peak was completely suppressed in the presence of IGF-I (Fig. 3*C*), and cells accumulated in S phase. The percentages of cells in S phase in Fig. 3*A* (untreated cells) and Fig. 3*C* (etoposide + IGF-I) were 11 and 25%, respectively.

The etoposide-induced appearance of internucleosomal low-molecular-weight DNA fragments is shown in Fig. 4*A* (*lane B*). In conjunction with the prevention of cell death, IGF-I partially inhibited

DNA fragmentation (Fig. 4*A*, *lane C*). These results corresponded to the prevention by IGF-I of the release of low-molecular-weight [^{14}C]thymidine-labeled DNA into the medium following exposure to etoposide, as shown in Fig. 4*B*.

We next tested whether a similar protective action of IGF-I could be elicited in either parental BALB/c3T3 cells (approximately 2×10^5 IGF-I-binding sites/cell) or cells devoid of the IGF-I receptor gene (R^- cells) derived from mouse embryos with targeted disruption of the IGF-I receptor, as described elsewhere (6). As shown in Fig. 5, parental BALB/c3T3 cells were effectively killed by etoposide (Fig. 5*A*, *lane 2*) but with less efficiency than p6 cells (Fig. 5*B*, *lane 2*), presumably because of the longer doubling time of BALB/c3T3 cells



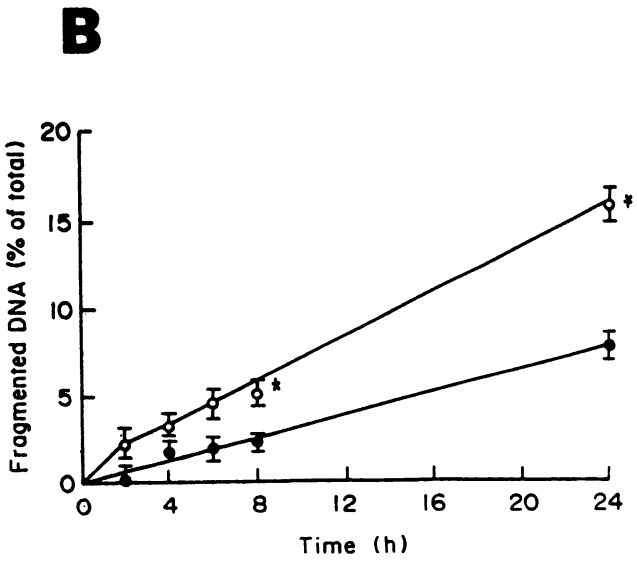


Fig. 4. Internucleosomal DNA fragmentation associated with etoposide-induced apoptosis. *A*, P6 cells were treated with etoposide (20 μM) either with (*lane C*) or without (*lane B*) IGF-I (50 ng/ml). *Lane A*, control cells without any addition. After 24 h, cytosolic DNA was purified from 10^6 cells and visualized by agarose electropheresis, as described "Materials and Methods." *m*, DNA molecular weight marker number VIII (Boehringer Mannheim); 501 and 242 base pairs are indicated. *B*, [^{14}C]thymidine-labeled p6 cells were treated with etoposide either with (●) or without (○) IGF-I. At the indicated times, cytosolic, released, and nuclear DNA were isolated, and the radioactivity in each fraction was determined by liquid scintillation counting, as described "Materials and Methods." *Points* (*bars*) are the means (±SE) of triplicate determinations from a representative experiment and represent the percentages of total DNA of the combined cytosolic and released DNA fractions. *, $P < 0.05$ compared to etoposide alone.

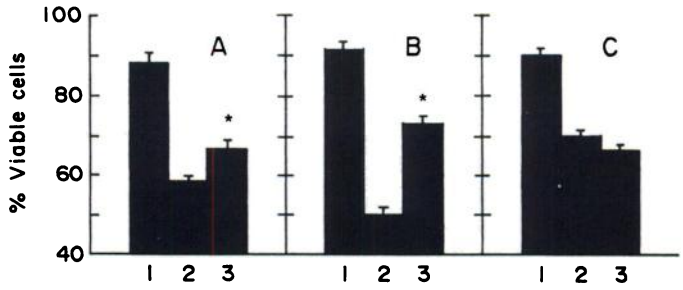


Fig. 5. Activated IGF-I receptor inhibits etoposide-induced cell killing. Cells (5×10^3 each) of BALB/c3T3 (*A*), p6 (*B*), or R^- (*C*) were cultured in the presence of serum for 16 h and then maintained in serum-free medium with either etoposide (20 μM; *lanes 2*) or etoposide + IGF-I (50 ng/ml; *lanes 3*) for an additional 24 h. *Lanes 1*, control cells without additions. *Columns* (*bars*) are the means (±SE) of triplicate determinations from a representative experiment and represent the percentages of viable cells. *, $P < 0.05$ compared to etoposide alone (*lanes 2*).

and the fewer number of these cells entering S phase. IGF-I was considerably less effective in preventing cell death in BALB/c3T3 cells (Fig. 5, *lanes 3*). R^- cells, which have the longest doubling time of the 3 lines tested, were the most resistant to etoposide. IGF-I had no effect on the killing of R^- cells by etoposide (Fig. 5*C*).

DISCUSSION

The enhanced survival of cells in culture could be due to a direct inhibition of apoptosis or, alternatively, a reflection of an increased proliferative rate. Apoptosis represents a fundamental intracellular program that is regulated both positively and negatively at various levels within the signaling pathways (see Ref. 27 for review). On one hand, apoptosis may be induced as a direct response to specific receptor activation (*e.g.*, tumor necrosis factor and glucocorticoid receptors) or toxic agents, such as chemotherapeutic agents and radiation. The susceptibility of a cell to undergo apoptosis under these conditions depends in part on growth factor-regulated intracellular signals that affect the progression of the apoptosis pathway. Alternatively, many cell types *in vitro* and *in vivo* undergo apoptosis simply when growth factors are removed (28). While a few protective intracellular regulators of apoptosis have been identified (27), the specific mechanisms by which growth factors prevent apoptosis need further exploration.

In the current study, we have demonstrated an important role for the IGF-I receptor as an antagonist of apoptosis, an effect that is independent of its proliferative action. The induction of S phase arrest in parental BALB/c 3T3 fibroblasts by etoposide stimulated apoptosis. IGF-I only slightly prevented cell death. However, the overexpression of the IGF-I receptor allowed for a potentiation of the action of IGF-I. Importantly, the survival of cells occurred despite a lack of mitogenesis.

These findings highlight a distinct survival function for the IGF-I receptor and, by association, its ligands IGF-I, IGF-II, and insulin. The role of IGF-I as a survival factor has been demonstrated previously most clearly in cells derived from the nervous system where it is required for maintenance of cultured cells and protection from direct injury (12–20). In particular, IGF-I is a trophic factor for cultured oligodendrocytes but does not induce their proliferation (13). IGF-I may function to replace other trophic growth factors required for cell survival. For example, IGF-I can inhibit apoptosis of several interleukin 3-dependent cell lines when interleukin 3 is removed (29). The current study of the IGF-I receptor is also in keeping with other emerging studies that demonstrate prevention of cell death by IGF-I in

response to direct activators of apoptosis, including tumor necrosis factor-α and radiation,[4] and deregulated c-*myc* expression (30).

The mechanism by which IGF-I prevents apoptosis remains to be clarified. At one level, the relative weakness of IGF-I in preventing apoptosis at low levels of the IGF-I receptors, and its complete ineffectiveness in cells devoid of IGF-I receptors, is reminiscent of its mitogenic action. For IGF-I-sensitive cells, growth factors such as platelet-derived growth factor and epidermal growth factor promote the entry from cell cycle G_0 phase to G_1 phase, but the interaction of IGF-I (or IGF-II and insulin) with the IGF-I receptor is necessary for the transition from G_1 to S phase (2). Competence factors act, at least in part, by increasing the number of IGF-I receptors. Indeed, IGF-I alone induces mitogenesis in cells that overexpress the IGF-I receptor (31–33). It should be noted, however, that the concentrations of IGF-I required to inhibit apoptosis are considerably higher than for mitogenesis (31).

The observation that IGF-I may function as a survival factor in response to diverse agents suggests that it blocks a common late intracellular apoptosis pathway. The nature of these putative molecules is not known, although our preliminary data suggest that bcl-2 mRNA is not increased in p6 cells in response to IGF-I (data not shown). In view of the observations that cell cycle arrest in general promotes apoptosis and that several cell cycle-related molecules may participate in the apoptosis program (34, 35), the diverse biological functions of IGF-1 may be regulated by a common set of IGF-I receptor-mediated signals.

The IGF-I receptor has emerged as a critical component of the transformation process (6, 33, 36). These findings, in conjunction with the current ones, have important potential implications for the clinical behavior of tumors and their therapy. Many malignant tumors secrete IGF-I and IGF-II and overexpress IGF-I receptors (37). Insofar as tumor growth depends in part on the balance of cell death versus proliferation, the autocrine/paracrine pathway of IGF-I stimulation is significant. However, we suggest that the most critical determinant of biological behavior is the level of the IGF-I receptor. The extent of IGF-I receptor expression may also play a role in the response of tumors to chemotherapeutic therapy and radiation. Studies are in progress in this laboratory (33) and others (36) to modify the biological behavior of tumors as well as their sensitivity to therapeutic agents by directly manipulating the levels of the IGF-I receptor.

REFERENCES

1. Sara, V. R., and Hall, K. Insulin-like growth factors and their binding proteins. Physiol. Rev., *70:* 591–614, 1990.
2. Baserga, R., and Rubin, R. Cell cycle and growth control. Crit. Rev. Euk. Gene Exp., *3:* 47–61, 1993.
3. Lowe, W. L., Jr. Biological actions of the insulin-like growth factors. *In:* D. LeRoith (ed.), Insulin-like Growth Factors: Molecular and Cellular Aspects, pp. 49–85. Boca Raton, FL: CRC Press, 1991.
4. DeChiara, T. M., Efstratiadis, A., and Robertson, E. J. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature (Lond.), *345:* 78–80, 1990.
5. Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J., and Efstratiadis, A. Mice carrying null mutations of the genes encoding insulin-like growth factor 1 (IGF-I) and type 1 IGF receptor (IGF1r). Cell, *75:* 59–72, 1993.
6. Sell, C., Rubini, M., Rubin, R., Liu, J., Efstradiadis, A., and Baserga, R. Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type-1 IGF receptors. Proc. Natl. Acad. Sci. USA, *90:* 11217–11221, 1993.
7. Sell, C., Dumenil, G., Deveaud, C., Miura, M., Coppola, D., DeAngelis, T., Rubin, R., Efstratiadis, A., and Baserga, R. Effect of a null mutation of the type-1 IGF receptor gene on growth and transformation of mouse embryo fibroblasts. Mol. Cell. Biol., *14:* 3604–3612, 1994.
8. Goldring, M. B., and Goldring, S. R. Cytokines and cell growth control. Crit. Rev. Eukaryotic Gene Expr., *1:* 301–326, 1991.
9. Huang, S., and Terstappen, L. W. M. M. Formation of haematopoietic microenvironment and haematopoietic stem cells from single human bone marrow stem cells. Nature (Lond.), *360:* 745–749, 1992.
10. Rodriguez-Tarduchy G., Collins, M. K. L., Garcia, I., and Lopez-Rivas A. Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. J. Immunol., *149:* 535–540, 1992.
11. Drago, J., Murphy, M., Carroll, S. M., Harvey, R. P., and Bartlett, P. F. Fibroblast growth factor-mediated proliferation of central nervous system precursors depends on endogenous production of insulin-like growth factor I. Proc. Natl. Acad. Sci. USA, *88:* 2199–2203, 1991.
12. McMorris, F. A., and Dubois-Dalcq, M. Insulin-like growth factor 1 promotes cell proliferation and oligodendrial commitment in rat glial progenitor cells developing in vitro. J. Neurosci. Res., *21:* 199–209, 1988.
13. Barres, B. A., Hart, I. K., Coles, H. S. R., Burne, J. F., Voyvodic, J. T., Richardson, W. D., and Raff, M. C. Cell death and control of cell survival in the oligodendrocyte lineage. Cell, *70:* 31–46, 1992.
14. Bozyczko-Coyne, D., Glicksman, M. A., Prantner, J. E., McKenna B., Connors, T., Friedman, C., Dasgupta, M., and Neff, N. IGF-I supports the survival and/or differentiation of multiple types of central nervous system neurons. Ann. N. Y. Acad. Sci., *692:* 311–313, 1993.
15. Svrzic, D., and Schubert, D. Insulin-like growth factor 1 supports embryonic nerve cell survival. Biochem. Biophys. Res. Commun., *172:* 54–60, 1990.
16. Raff, M. C., Barres, B. A., Burne, J. F., Coles, H. S., Ishizaki, Y., and Jacobson, M. D. Programmed cell death and the control of cell survival: lessons form the nervous system. Science (Washington DC), *262:* 695–700, 1993.
17. Gluckman, P., Klempt, N., Guan, J., Mallard, C., Sirimanne, E., Dragunow, M., Klempt, M., Singh, K., Williams, C., and Nikolics, K. A role for IGF-I in the rescue of CNS neurons following hypoxic-ischemic injury. Biochem. Biophys. Res. Commun., *182:* 593–599, 1992.
18. Calissano, P., Ciotti, M. T., Battistini, L, Zona, C., Angenlini, A., Merlo, D., and Mercanti, D. Recombinant human insulin-like growth factor I exerts a trophic action and confers glutamate sensitivity on glutamate-resistant cerebellar cells. Proc. Natl. Acad. Sci. USA, *90:* 8752–8756, 1993.
19. D'Mello, S. R., Galli, C., Ciotti, T., and Calissano, P. Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. Proc. Natl. Acad. Sci. USA, *90:* 10989–10993, 1993.
20. Cheng, B., and Mattson, M. P. IGF-I and IGF-II protect cultured hippocampal and septal neurons against calcium mediated hypoglycemic damage. J. Neurochem., *12:* 1558–1566, 1992.
21. Schwartzman, R. A., and Cidlowski, J. A. Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocr. Rev., *14:* 133–151, 1993.
22. Collins, M. K. L., Perkins, G. R., Rodriguez-Tarduchy, G., Nieto, M. A., and Lopez-Rivas, A. Growth factors as survival factors: regulation of apoptosis. Bioessays, *16:* 133–138, 1994.
23. Lammers, R., Gray, A., Schlessinger, G., and Ullrich, A. Differential signalling potential of insulin- and IGF1-receptor cytoplasmic domains. EMBO J., *8:* 1369–1375, 1989.
24. Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Katchurea, S., Chen, E., Jacobs, S., Francke, D., Ramachandran, J., and Fugita-Yamaguchi, Y. Insulin-like growth factor 1 receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J., *5:* 2503–2512, 1986.
25. Wang, J. C. DNA topoisomerases. Annu. Rev. Biochem., *54:* 665–697, 1985.
26. Darzynkiewick, Z., Bruno, S., Del Bino, G., Gorczyca, W., Hotz, M. A., Lassota, P., and Traganos, F. Features of apoptotic cells measured by flow cytometry. Cytometry, *13:* 795–808, 1992.
27. Williams, G. T., and Smith, C. A. Molecular regulation of apoptosis: genetic controls on cell death. Cell, *74:* 777–779, 1993.
28. Collins, M. K. L., Perkins, G. R., Rodriguez-Tarduchy, G., Nieto, M. A., and Lopez-Rivas, A. Growth factors as survival factors: regulation of apoptosis. Bioessays *16:* 133–138, 1994.
29. Rodriguez-Tarduchy, G., Collins, M. K. L., Garcia, I., and Lopez-Rivas, A. Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. J. Immunol., *149:* 535–540, 1992.
30. Harrington, E. A., Bennett, M. R., Fanidi, A., and Evan, G. I. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytolines. EMBO J., *13:* 3286–3295, 1994.
31. Pietrzkowski, Z., Lammers, R., Carpenter, G., Soderquist, A. M., Limardo, M., Phillips, P. D., Ullrich, A., and Baserga R. Constitutive expression of insulin-like growth factor 1 and insulin-like growth factor 1 receptor abrogates all requirements for exogenous growth factors. Cell Growth Differ., *3:* 199–205, 1992.
32. Ambrose, D., Resnicoff, M., Coppola, D., Sell, C., Miura, M. Jameson, S., Baserga, R., and Rubin, R. Growth regulation of human glioblastoma T98G cells by insulin-like growth factor-1 (IGF-1) and the IGF-1 receptor. J. Cell. Physiol., *159:* 92–100, 1994.
33. Resnicoff M., Sell, C., Rubini, M., Coppola, D. Ambrose, D., Baserga, R., and Rubin, R. Rat glioblastoma cells expressing an antisense RNA to the insulin-like growth factor-1 (IGF-1) receptor are non-tumorigenic and induce regression of wild-type tumors. Cancer Res., *54:* 2218–2222, 1994.
34. Meikrantz, W., Gisselbrecht, S., Tam, S. W., and Schlegel, R. Activation of cyclin A-dependent protein kinases during apoptosis. Proc. Natl. Acad. Sci. USA, *91:* 3754–3758, 1994.
35. Fath, I., Schweighoffer, F., Rey, I., Multon, M. C., Boiziau, J., Duchesne, M., and Tocque, B. Cloning of Grb2 isoform with apoptotic properties. Science (Washington DC), *264:* 971–974, 1994.
36. Prager, D., Li, H. L., Asa, S., and Melmed, S. Dominant negative inhibition of tumorigenesis *in vivo* by human insulin-like growth factor I receptor mutant. Proc. Natl. Acad. Sci. USA, *91:* 2181–2185, 1994.
37. Macaulay V. M. Insulin-like growth factors and cancer. Br. J. Cancer, *65:* 311–320, 1992.

[4] C. Sell, R. Baserga, and R. Rubin, manuscript in preparation.