Reversibility of Serum Removal Effects on IGF-II mRNA in Human Neuroblastoma Cells

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Insulin-like growth factor II (IGF-II) is highly expressed at the mRNA and protein levels in SH-SY5Y human neuroblastoma cells.[1,2] SH-SY5Y cells also express type I and type II IGF receptors,[2,4] and respond to IGF-II with increased DNA synthesis and neurite outgrowth,[4] suggesting the presence of an IGF-II-mediated autocrine growth mechanism. Based on these results, we hypothesized that the rate of SH-SY5Y cell proliferation is partly determined by the level of IGF-II gene expression, and that IGF-II expression may be serum dependent. We tested this hypothesis using northern analysis to determine the effects of removal and subse-

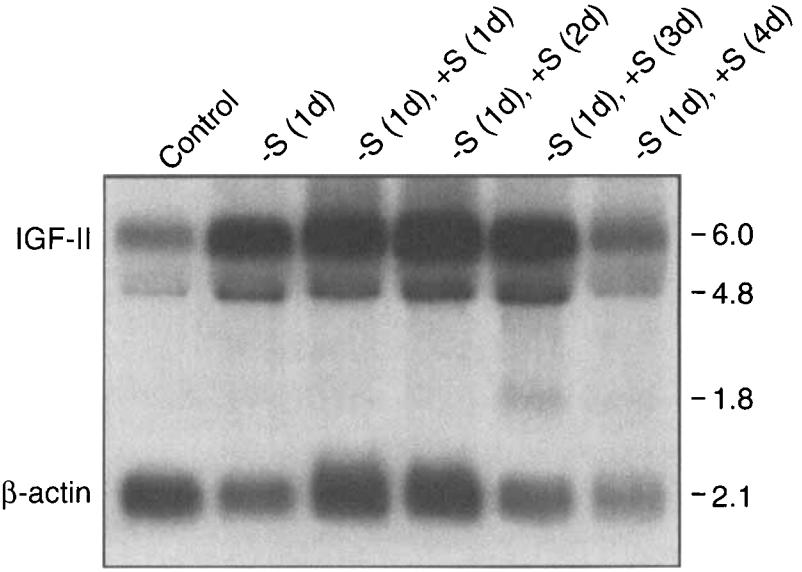

FIGURE 1. Northern analysis of IGF-II mRNA. SH-SY5Y cells were plated (3.7×10^6 cells/cm^2 in T150 flasks) and cultured for 2 days in 10% calf serum and DMEM. Cells were rinsed with DMEM, and DMEM added. After 1 day, the medium was removed and DMEM + 10% calf serum added. RNA was isolated either before serum removal (no rinse), 1 day after serum (S) deprivation, or 1, 2, 3, or 4 days after transfer back to serum, as indicated in the legend. Total RNA was electrophoresed and northern analysis performed as described,[2] with ^{32}P-cDNA labeled probes for human IGF-II and β-actin.

quent addition of serum on cultured SH-SY5Y cell IGF-II mRNA levels. RNA was analyzed from cells that were serum deprived for one day or cells that were serum deprived and subsequently transferred to serum-containing media for one to four days.

SH-SY5Y cells expressed three IGF-II mRNA transcripts (1.8, 4.8, and 6.0 kb), of which the 6.0-kb transcript was the most abundant, as previously described.[2] A 2.1-kb β-actin mRNA was also detected, and served as a control for the amount of

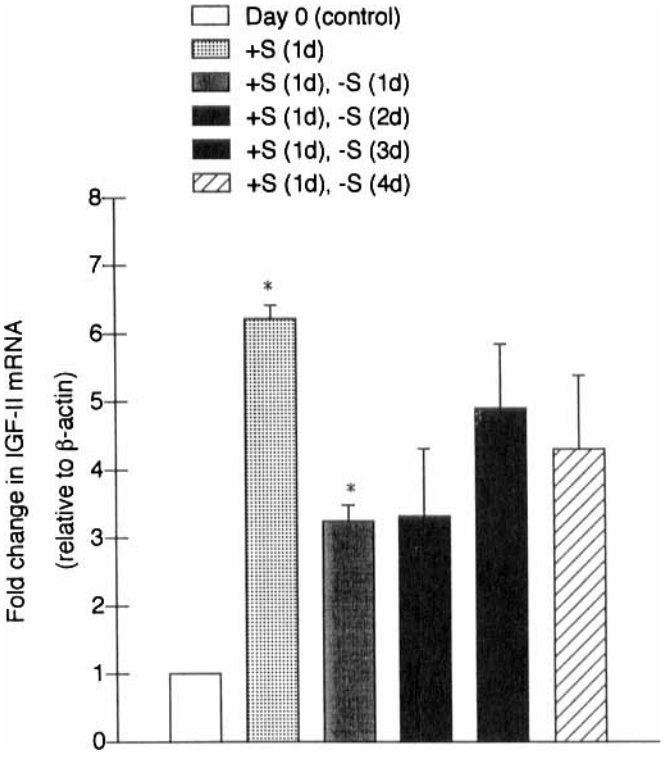

FIGURE 2. Densitometric analysis of IGF-II mRNA. Optical densities of the 6.0-kb IGF-II and 2.1 kb β-actin bands for each lane of several autoradiographs like that shown in FIGURE 1 were densitometrically quantitated, and IGF-II mRNA expressed as a percentage of the untreated control relative to β-actin. $*p < 0.05$ relative to untreated, day 0 control levels by unpaired, two-tailed *t*-test.

RNA loaded onto the gel. Levels of the 6.0-kb IGF-II mRNA increased by 6-fold after a one-day serum deprivation (FIGURES 1 and 2, lanes 1 and 2). One day following the subsequent addition of serum to SH-SY5Y cells, IGF-II mRNA was reduced to 3-fold above untreated control levels (FIGURES 1 and 2, lanes 1 and 3). Two, three, or four days after subsequent serum addition, SH-SY5Y cell IGF-II mRNA levels were not significantly different from untreated control levels (FIGURES 1 and 2, compare lane 1 with lanes 3, 4, 5, and 6).

These observations indicate that steady-state IGF-II mRNA levels increase with serum removal and are partially reversed by the addition of serum. IGF-II mRNA transcripts in stably transfected mouse cells are differentially expressed in a serum-dependent fashion.[3] We observed no changes in the relative abundance of the three IGF-II mRNA transcripts in human SH-SY5Y cells, suggesting that species-specific (mouse vs. human) factors may be important in the regulation of IGF-II gene expression.

In summary, we found that IGF-II mRNA levels in SH-SY5Y cells are increased with serum deprivation, and partially reversed to untreated control levels by subsequent addition of serum-containing media. Regulation of IGF-II gene expression by serum may be associated with changes in SH-SY5Y cell growth. Indeed, we have shown elsewhere that IGF-II gene expression is decreased with growth inhibition by the cytokine interferon-γ.[1]

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