

# Insulin-like growth factors I and II induce cell death in Wilms's tumour cells

M Granérus, A Johannisson, P Ekblom, W Engström

## Abstract

**Aim**—To study the effects of insulin-like growth factors (IGFs) on the growth phenotype of a Wilms's tumour cell line (WCCS-1).

**Methods**—WCCS-1 cells were cultured in vitro and exposed to IGF-I and IGF-II, as well as their antagonists, IGF binding protein 2 and the type I receptor blocking antibody IGF-IR $\alpha$ . The effects on proliferation and cell cycle parameters were assayed by assessing cell numbers, autoradiography after labelling with tritiated thymidine, and flow cytometry after double staining with fluorescein isothiocyanate (FITC) labelled annexin V and propidium iodide.

**Results**—The addition of IGF-I as well as IGF-II in physiological doses induced cell death in Wilms's tumour cells. Cell numbers decreased most dramatically on the fifth to sixth day after growth factor addition. The occurrence of apoptosis as well as necrosis was confirmed by annexin-V staining of cell cultures. S-phase indices were comparable, irrespective of whether the cells were exposed to IGFs or not, which suggests that WCCS-1 cells undergo cell death at random during the cell cycle rather than from the prereplicative phase. To exclude any influences of the IGF binding proteins (IGFBPs), all results were repeated with Des(1-3)IGF-I, which is unable to bind to any of the IGFBPs. However, this peptide was equally potent in inducing cell death. Finally, the addition of IGFBP-2 or the type 1 receptor blocking antibody IGF-IR $\alpha$  partly abrogated the death inducing effects of IGF-I and IGF-II.

**Conclusions**—Insulin like growth factors induce cell death—apoptosis as well as necrosis—in cultured Wilms's tumour cells. Furthermore, it is proposed that this effect is mediated by the type 1 receptor.

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**Keywords:** insulin-like growth factor I; insulin-like growth factor II; Wilms's tumour; apoptosis

locus associated with Wilms's tumour led to the cloning and characterisation of the gene, WT1, which encodes a zinc finger DNA binding protein that functions as a transcriptional repressor.<sup>3,4</sup> One of the first actions revealed for WT1 was its repression of the insulin-like growth factor II (IGF-II) gene. Wilms's tumours are in general characterised by a high rate of expression of the IGF-II gene.<sup>5,6</sup> IGF-II is known to be a ubiquitous and essential growth factor during embryogenesis,<sup>7,8</sup> and acts as a survival factor in embryonic tumour cell lines.<sup>9-11</sup> Taken together, these data suggested a growth regulatory loop whereby IGF-II supports either proliferation or survival and WT1 counteracts this by specifically suppressing IGF-II transcription. In this report we challenge the universal applicability of this concept. Wilms's tumour cells grown in vitro respond to either of the IGFs by an increased rate of cell death. Even though growth inhibitory effects have been recorded previously for IGF-II,<sup>12</sup> and IGF II can act as a tumour suppressor in vivo, we show for the first time that both IGFs can induce cell death—in one Wilms's tumour derived cell line at least—rather than unequivocally acting as a survival factor for tumour cells.

## Material and methods

### CELL CULTURE

The WCCS-1 cell line was maintained and flasks and wells for experimental purpose were prepared essentially as described by Talts *et al.*<sup>13</sup> All tissue culture material was obtained from NUNC (Roskilde, Denmark) and GIBCO (through Life Technologies, Paisley, UK).

The serum free medium consisted of a 1/1 (vol/vol) mixture of  $\alpha$  medium lacking nucleosides and deoxynucleosides and Ham's F12 medium supplemented with 10  $\mu$ g/ml of human transferrin preloaded with iron, in accordance with the manufacturer's instructions. This medium is subsequently called  $\alpha$ ;Ham.

Two days before a growth experiment the medium over the stock cultures was replaced by  $\alpha$ ;Ham supplemented with 10% heat inactivated fetal calf serum (FCS). This procedure allowed the cells to adapt to the basal medium that was used in the serum free culture experiments. The cells were removed from the stock culture flasks by rinsing with phosphate buffered saline (PBS) and then briefly exposing the cells to a mixture of trypsin, versene, and chick plasma (TVP). The cells were taken up in  $\alpha$ ;Ham with 10% FCS, spun once, resuspended in  $\alpha$ ;Ham with 10% FCS, and counted in a Coulter counter (Coulter Electronics Ltd, Bedford, UK).

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Wilms's tumour is a childhood neoplasm that is believed to develop when multipotent kidney blastemal cells fail to differentiate and continue to proliferate after birth.<sup>1</sup> The occurrence of sporadic as well as hereditary variants of Wilms's tumour and the early age of bilateral kidney tumour onset suggested that Wilms's tumours developed when a predisposing germ line mutation was accompanied by a second mutation or loss of heterozygosity at the disease locus.<sup>2</sup> Fine mapping of the chromosomal

In the subsequent cell culture experiments, the media were pre-equilibrated for at least six hours in an atmosphere of 5% (vol/vol) CO<sub>2</sub> in 95% humidified air at 37°C. The day before the experiment, the cells were plated out in 10 ml of  $\alpha$ -Ham with 10% FCS at a density that varied between  $1.4 \times 10^5$  and  $3.0 \times 10^5$  cells/60 mm diameter Primaria dish (Becton Dickinson, San Jose, California, USA). The number of cells plated out varied between experiments but numbers were always comparable within each experiment. At the start of each experiment, the cells were rinsed twice in PBS and 10 ml of  $\alpha$ -Ham was then added to each dish. During the next hour the dishes were briefly removed from the incubator and the various experimental compounds were added to the dishes. Two dishes were counted immediately after the PBS rinse to obtain a starting cell count, and on subsequent days the effect of the additives was measured by counting duplicate dishes in each treatment. For these counts, the cells were exposed to trypsin (0.125%, wt/vol) and EDTA (wt/vol) in PBS for up to 30 minutes to ensure complete detachment from the dish surface. When all cells had detached, any remaining trypsin was neutralised by adding soybean trypsin inhibitor. The cells were counted in triplicate in a Coulter counter. The range of the cell counts of the duplicate dishes rarely exceeded 8% of the mean cell count.

#### GROWTH FACTORS AND OTHER ADDITIVES

Recombinant IGF-I and IGF-II were purchased from British Biotechnology, UK. Modified Des(1-3)IGF-II was obtained from GroPep (Adelaide, Australia). Bovine IGF binding protein 2 (IGFBP-2) was purchased from GroPep, and the IGF-IR $\alpha$  (1H7) was obtained from Santa Cruz Biotechnology (Santa Cruz, California, USA).

The lyophilised IGF-I and IGF-II samples were made up to stocks of 2  $\mu$ g/ml by adding 0.5 ml of 0.1 M acetic acid to 10  $\mu$ g, shaking every five minutes in a 37°C water bath, then adding 4 ml of PBS with 1% wt/vol crystalline bovine serum albumin (BSA; Sigma, Stockholm, Sweden), adjusting the pH to 7.0 with 0.1 M NaOH, and finally bringing the volume up to 5 ml with PBS/BSA. These stocks were aliquoted into Eppendorf vials and stored at -70°C until use.

The IGFBP-2 was dissolved in sterile filtered 10 mM HCl at a concentration of 100  $\mu$ g/ml, snap frozen in liquid nitrogen, and stored until further use. The anti-IGF-IR $\alpha$  (1H7) antibody, which specifically recognises the IGF-I receptor type 1 $\alpha$  subunit, and thereby inhibits binding of both IGF-I and IGF-II to this receptor was supplied in the form of 200  $\mu$ g IgG dissolved in 1 ml of PBS with 0.1% sodium azide and 0.2% gelatin. This vial was kept at 4°C.

In all growth experiments, growth factor was added on a daily basis at the same concentration as stated in the tables and figures. Control dishes were supplemented with either 5% FCS or with a dose of BSA, at the same concentration as that used as a vehicle for the growth factor. This procedure ensured that the control dishes were exposed to the same degree of cooling and the same pH changes.

MEASUREMENT OF ANNEXIN V STAINING AND PI INCORPORATION WITH FLOW CYTOPHOTOMETRY  
Cell suspensions were prepared for flow cytometric analysis from cultures growing in: (1)  $\alpha$ -Ham, (2)  $\alpha$ -Ham with 10% FCS, (3)  $\alpha$ -Ham with 30 ng/ml IGF-I, or (4)  $\alpha$ -Ham with 30 ng/ml IGF-II. Cell suspensions containing 500 000 cells were collected in the following way. Dishes were rinsed five times in prewarmed 37°C PBS to remove any detached cells. The remaining cells were trypsinised by a quick treatment with TVP as described above and then taken up in 10% FCS. The cells were spun down and resuspended in 500  $\mu$ l binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl<sub>2</sub>). Of this suspension, 100  $\mu$ l was transferred to a 5 ml tube. Aliquots of 5  $\mu$ l of annexin V-fluorescein isothiocyanate (FITC) (Pharmingen, San Diego, California, USA) and 10  $\mu$ l of propidium iodide (PI; 50  $\mu$ g/ml stock solution; Pharmingen) were added, and the cells were incubated for 15 minutes at room temperature in the dark. Then, 400  $\mu$ l of binding buffer was added to each tube, and the samples were analysed. Controls (unstained cells, cells stained with annexin V (but not with PI), cells stained with PI (but not with annexin V)) were also prepared. The flow cytometer used was a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, California, USA). List-mode files were collected containing data for size (FSC-H), granularity (SSC-H), annexin V-FITC (FL1-H), and PI (FL3-H) for 10 000 events from each sample. Debris and aggregates were excluded using size and granularity and the percentages of (1) unstained cells, (2) annexin V positive but PI negative cells, (3) annexin V negative but PI positive cells, and (4) annexin V and PI positive cells were obtained. Irradiated human lymphocytes were repeatedly examined and the results used as a control of the reliability of the method.

#### AUTORADIOGRAPHY

WCCS-1 cells were grown on NUNC plastic 24 well plates and pulse labelled for one hour with 10  $\mu$ Ci tritiated thymidine/well (56 Ci/mmol; Amersham, Little Chalfont, Buckinghamshire, UK). After labelling, the cells were briefly rinsed in ice cold PBS and fixed in methanol/acetic acid (3/1; vol/vol) for one hour. The cells were then exposed to aqueous 10% (wt/vol) trichloroacetic acid for 30 minutes at 4°C to remove free radioisotope and then washed three times in distilled water. The dishes were then coated with Ilford K2 emulsion, and exposed for five to seven days at 4°C, essentially as described by Hyldahl *et al.*<sup>14</sup> The proportion of labelled cells was recorded by counting at least 1000 cells using the light microscope.

#### Results

Table 1 shows the effects of the various growth factors on the multiplication of WCCS-1 cells. Cells growing in 10% serum had approximately trebled in number after five days. This figure differs slightly from those obtained in previous studies<sup>13</sup> but was nevertheless

Table 1 The effect of growth factor addition on cell numbers

Day	SFM	10% FCS	IGF-I	IGF-II
1	165 (28)	165 (28)	165 (28)	165 (28)
4	204 (49)	410 (99)	136 (38)	192 (40)
5	189 (55)	586 (88)	111 (19)	103 (26)
6	175 (42)	612 (109)	86 (59)	79 (27)

Cells were seeded in  $\alpha$ Ham supplemented with 10% FCS on day 0. On day 1 each culture was rinsed and  $\alpha$ Ham with supplements (10% FCS, 30 ng/ml IGF-I or IGF-II) was added. To ensure a continuous supply of growth factors, serum or the appropriate growth factor was added daily. Cells were counted in triplicate on a Coulter counter on the days indicated in the table. Each figure is based on two different experiments and the data expressed as mean (SD).

FCS, fetal calf serum; IGF, insulin-like growth factor; SFM, serum free medium.

obtained during repeated experiments. In contrast, when the cells were grown in the absence of macromolecular additives, the increase in cell numbers was retarded during the first four to five days, but only after five days in the absence of serum or other growth promoting substances did cell numbers begin to decrease. When WCCS-1 cells were cultured in the presence of 30 ng/ml IGF-I or IGF-II, there was a conspicuous drop in cell numbers by the fourth day in culture. After five or six days, in the presence of IGF-I or IGF-II cell numbers were far below those seen in cultures grown in serum free medium (table 1). Even low concentrations of IGF-I or IGF-II (1 ng/ml medium) exerted an observable repressive effect on cell numbers. This suppression of cell numbers increased as the concentration of IGFs increased. Figure 1 shows the morpho-

logical appearance of the cells in the presence or absence of IGFs. It is clearly shown that the cellular morphology does not change with changing culture conditions. The cells retain their polygonal shape and exhibited a clear epithelial-like morphology.

To exclude any spurious effect exerted by any of the circulating IGF binding proteins (IGFBPs), we examined the effects of a modified IGF-I molecule (Des(1-3)IGF-I), which binds to the type 1 receptor with unaltered affinity but not to any of the IGFBPs. This modified growth factor was just as potent as IGF-I and IGF-II in causing a decrease in cell numbers. To pursue this issue further, we concomitantly exposed WCCS-1 cells to IGF-I or IGF-II and an excess of IGFBP-2. Table 2 shows that the presence of the binding protein abrogated the inhibitory action of IGF-I or IGF-II on cell numbers. Similarly, the effect of IGF-I or IGF-II on cell numbers could be counteracted by the presence of anti-IGF-IR $\alpha$ , which is an antibody that specifically recognises the  $\alpha$  subunit of the IGF type 1 receptor. However, it was unclear whether the addition of either of the IGFs forced cells to leave the cell cycle and to enter G0. To elucidate whether this was the case, we pulse labelled cells after 72 hours with tritiated thymidine, to obtain a measure of the proportion of cells traversing the cell cycle (table 3). It was found that, irrespective of culture conditions, the thymidine labelling indices varied only between 24% and 29%, which suggests that in each of these scenarios, the surviving WCCS-1 cells

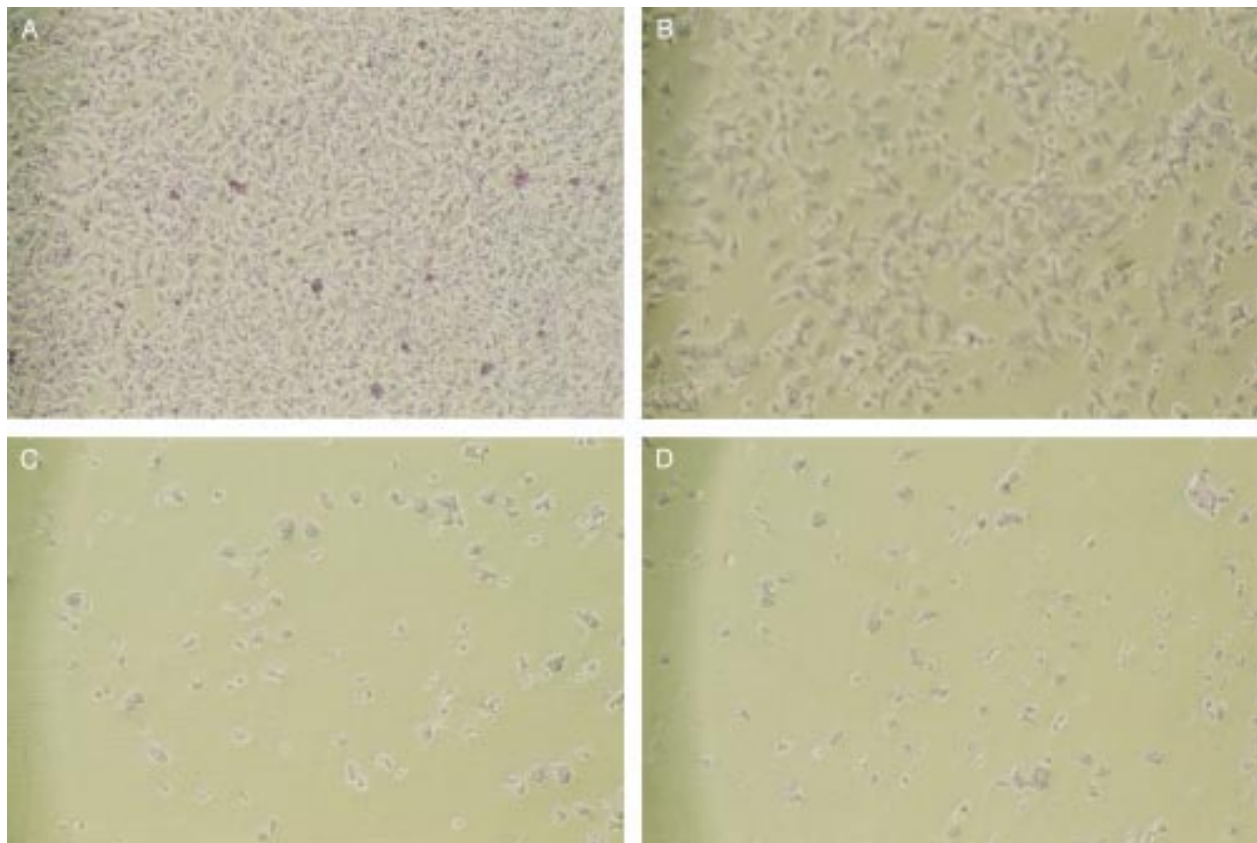


Figure 1 Morphological appearance of WCCS-1 cells grown for five days in (A) 10% serum, (B) serum free medium (SFM) (C) SFM + 30 ng/ml insulin-like growth factor I (IGF-I), and (D) SFM + 30 ng/ml IGF-II.

Table 2 The effect of IGF antagonists on cell numbers

Day	SFM	10% FCS	IGF-I	IGF-I + IGFBP-2	IGF-I + IGF-IRa
1	165 (28)	165 (28)	165(28)	165 (28)	165 (28)
4	204 (49)	410 (99)	136(38)	355 (71)	399 (87)
5	189 (55)	586 (88)	111(19)	381 (55)	312 (26)
6	175 (42)	612 (109)	86(59)	361 (39)	401 (98)

Cells were seeded in  $\alpha$ ;Ham supplemented with 10% FCS on day 0. On day 1 each culture was rinsed and  $\alpha$ ;Ham with the appropriate additives was added (10% FCS, 30 ng/ml IGF-I, 50 ng/ml IGFBP-2, 20 ng IGF-IRa antibody). Serum or the appropriate supplement was added daily to ensure a continuous supply in the cell cultures. Cells were counted in triplicate in a Coulter counter on the days indicated in the table. Each figure is based on two different experiments and expressed as mean (SD).

FCS, fetal calf serum; IGF, insulin-like growth factor; IGFBP, IGF binding protein; IGF-IRa, anti-IGF-I receptor Ia antibody; SFM, serum free medium.

Table 3 The effect of growth factor addition on DNA synthesis in WCCS-1 cells

Medium	% Labelled cells
10% serum	28.5 (7.4)
$\alpha$ ;Ham (SFM)	29.1 (5.6)
SFM + 10 ng IGF-I/ml	26.6 (8.1)
SFM + 10 ng IGF-II/ml	29.7 (6.0)
SFM + 10 ng Des(1-3)IGF-I/ml	27.0 (9.6)

Values are mean (SD).

Cells were seeded at sparse density and grown in  $\alpha$ ;Ham supplemented with 10% serum for 24 hours and then rinsed in phosphate buffered saline and exposed to the experimental media for 72 h. Between the 71st and 72nd hour, the cultures were pulse labelled with 10  $\mu$ Ci tritiated thymidine/ml medium. The cultures were fixed and subjected to autoradiography. The proportion of cells labelled with tritiated thymidine was determined by light microscopy and expressed as percentages to adjust for the large difference in total cell numbers seen in the different experimental situations

IGF, insulin-like growth factor; SFM, serum free medium.

continue to proliferate at a similar rate and that any alterations of cell numbers result from a changing balance between cellular life and death.

Therefore, the IGF induced decrease in cell numbers was most probably the result of an increased rate of cell death. In an attempt to characterise the resulting cell death in more detail, we took advantage of a recently developed technique that distinguishes cells undergoing programmed cell death from necrotic cells.<sup>15</sup> In principle, one of the early changes of the plasma membrane during apoptosis is the translocation of phosphatidylserine from the inner layer of the plasma membrane to the outer layer. Annexin V is a calcium ion dependent phospholipid binding protein with high affinity for phosphatidylserine and can therefore be used as a probe for phosphatidylserine exposure on the cell membrane. Translocation of phosphatidylserine occurs during apoptosis as well as necrosis. The difference between these two forms of cell death is that during the initial stages of apoptosis the cell membrane remains intact whereas at the very moment that necrosis occurs the cell membrane loses its integrity and becomes leaky. Thus, annexin V binding to the cell surface must be viewed in conjunction with a dye exclusion test to establish the integrity of the cell membrane.<sup>15</sup> Annexin V binding was therefore assessed using bivariate flow cytometry and the cells stained with FITC labelled annexin V simultaneously with dye

exclusion of PI. This approach discriminates between intact cells (FITC-/PI-), apoptotic cells (FITC+/PI-), and necrotic cells (FITC+/PI+). We used cells grown for four days in the experimental media; that is, when the increase in cell death was first expected to be observed, and stained them with FITC-annexin V-PI. Figure 2 compares cells grown in serum free medium with those grown in 30 ng/ml IGF-I or IGF-II. In a control chart, cells grown in 10% serum showed nearly 90% intact cells. In the serum free media, with or without IGF addition, apoptotic as well as necrotic cells could be scored. However, when the effects of the two growth factors were compared, it was found that IGF-I exposure yielded a relatively larger proportion of apoptotic cells and IGF-II a relatively larger necrotic cell population.

## Discussion

The role of growth factors as multifunctional polypeptides is becoming increasingly diverse. Certain growth factors that normally support the proliferation of specific cells can act as harbingers of death when they bind to tumour cells. One early example of this dual role was demonstrated for the hepatocyte growth factor, which stimulates the proliferation of hepatocytes as well as endothelial cells and melanocytes, but possesses tumoricidal activity in a variety of other cell types.<sup>16</sup>

This study has shown that the addition of the insulin like growth factors IGF-I and IGF-II induces cell death in Wilms's tumour cells cultured in vitro. There was also a slight qualitative difference between the two growth factors because IGF-I appeared to exert a preferential effect on the induction of apoptosis, whereas IGF-II addition appeared to yield a relatively larger necrotic cell population. At first sight this observation is puzzling, but there are several recorded examples of inverse interactions between IGF-I and IGF-II,<sup>17</sup> and a possible explanation for their slightly different qualitative effects on cell death is that the two growth factors bind with different affinities to the two receptors—the signal transducing type 1 receptor and the endocytotic type 2 receptor. However, it was shown that blocking of the type 1 receptor abrogated the effect of IGF addition on cell death, indicating a key role of this receptor in the IGF induced lethal pathways.

The biological role of the IGFs is becoming increasingly complex. Whereas it is generally accepted that they play a pivotal role in embryonic growth and development,<sup>18,19</sup> most convincingly demonstrated by experimental disruption of the IGF-II gene in transgenic mice,<sup>6,7</sup> their role in tumour development and growth is less well understood.

In many naturally occurring and experimentally induced tumours, the fetal IGF-II promoters are reactivated. This was first seen in a group of developmental tumours including hepatoblastoma, embryonal nephroblastoma (Wilms's tumour), adrenocortical carcinoma, and rhabdomyosarcoma.<sup>5</sup> One feature that these tumours have in common is that the corresponding normal fetal tissues make large

quantities of IGF-II mRNA and protein.<sup>5 20</sup> By studying gene expression during fetal kidney formation it was concluded that the high expression of IGF-II by the blastema component of Wilms's tumour reflects the corresponding expression in normal blastema during the developmental window in which the tumour is thought to arise.<sup>21 22</sup> This evidence suggested for a long time that the growth and development of embryonic tumours was aided by an oncofetal paracrine loop in which IGF-II was a prime messenger. However, our study demonstrates that IGF-II (as well as IGF-I), rather than fulfilling their expected role as stimulants of cell proliferation and survival, in fact induced cell death in Wilms's tumour cells *in vitro*.

This finding is in line with two other pieces of evidence. First, there is a notable report where excess concentrations of IGF-II decreased skin sensory innervation in transgenic animals, thereby indicating a potential growth inhibitory role of IGF-II on a normal tissue *in vivo*.<sup>12</sup> Second, forced expression of IGF-II was found to prevent tumour formation in nude mice, which suggests a clear role of IGF-II as a tumour suppressor *in vivo*.<sup>23</sup> Moreover, there

are qualitative as well as quantitative differences in biological activity between bona fide IGF-II and larger variants that are sometimes produced by tumour cells.<sup>24</sup>

This novel role of IGF-II as a tumour suppressor conforms with earlier genetic data. Spontaneous as well as hereditary Wilms's tumour allele losses around the IGF-II locus (11p15.5) have been described.<sup>25 26</sup> On the other hand, imprinting of the IGF-II gene, which by itself limits the expression potential, has been shown to be relaxed in Wilms's tumours.<sup>27</sup> This paradox could possibly be explained by the operation of a dual mechanism that aims to reduce gene dosage as well as to regulate the production of bioactive IGF-II post-transcriptionally. A clear discrepancy between transcriptional activity and circulating concentrations of IGF-II protein in tumour tissue was in fact discovered more than a decade ago.<sup>28</sup>

The enhancement of cell death by both IGFs in the Wilms's tumour derived cell line WCCS-1 is the first of its kind to be recorded. However, it remains to be shown whether IGFs can induce cell death on cells from other

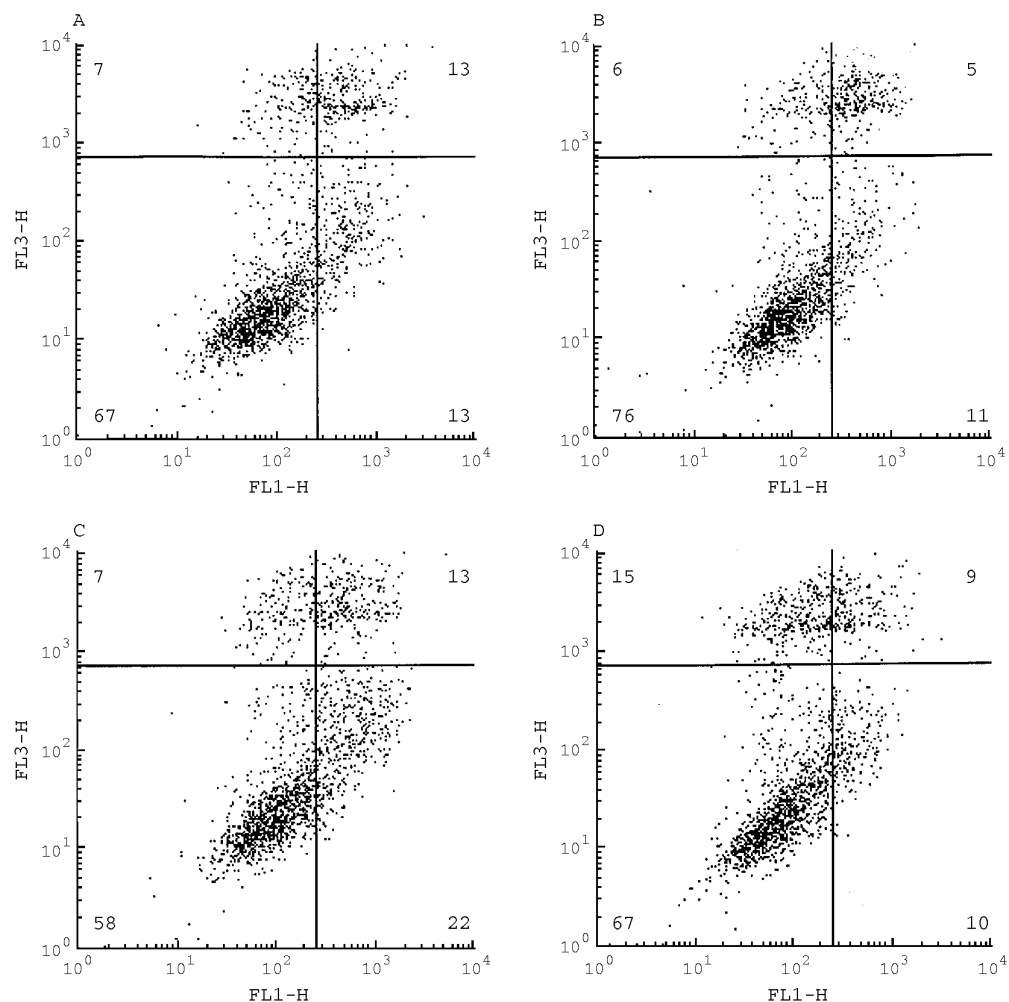


Figure 2 Flow cytometry data of staining with annexin V-fluorescein isothiocyanate (FITC) (abscissa) and propidium iodide (PI) (ordinate). WCCS-1 cells were seeded into 10% serum and then rinsed and exposed to either (A) serum free medium, (B) 10% serum, (C) 30 ng/ml insulin-like growth factor I (IGF-I), or (D) 30 ng/ml IGF-II. In each figure, the numbers in each compartment indicate the percentage of unstained cells (bottom left); annexin V positive, PI negative (bottom right); annexin V negative, PI positive (top left); and annexin V positive, PI positive (top right).

Wilms's tumours, as well as in other embryonic tumour cells. This work is currently in progress.

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- 1 Ward A. Beckmann-Wiedemann syndrome and Wilms tumour. *Mol Hum Reprod* 1997;3:157-68.
- 2 Knudson A, Strong LC. Mutation and cancer. A model for Wilms tumour of the kidney. *J Natl Cancer Inst* 1972;48:313-23.
- 3 Call KM, Glaser T, Ito CY. Isolation and characterisation of a zinc finger polypeptide gene at the human chromosome 11 Wilms tumour locus. *Cell* 1990;60:509-20.
- 4 Gessler M, Poustka A, Cavenee W, et al. Homozygous deletions in Wilms tumours of a zinc finger gene identified by chromosome jumping. *Nature* 1990;343:774-8.
- 5 Scott J, Cowell J, Robertson ME, et al. Insulin like growth factor II gene expression in Wilms tumours and embryonic tissues. *Nature* 1985;317:260-2.
- 6 Reeve AE, Eccles MR, Wilkins RJ, et al. Expression of insulin like growth factor II transcripts in Wilms tumour. *Nature* 1985;317:258-60.
- 7 de Chiara TM, Robertson EJ, Efstradiatis A. A growth deficiency phenotype in heterozygous mice carrying an insulin like growth factor II gene disrupted by gene targeting. *Nature* 1990;345:78-80.
- 8 de Chiara TM, Efstradiatis A, Robertson E. Parental imprinting of the mouse insulin like growth factor II gene. *Cell* 1991;64:849-59.
- 9 Granger M, Bierke P, Zumkeller W, et al. Insulin like growth factor II prevents apoptosis in a human teratoma derived cell line. *J Clin Pathol* 1995;48:M153-7.
- 10 Granger M, Bierke P, Schofield PN, et al. Growth factors and apoptosis in development. The role of insulin like growth factor I and TGF beta1 in regulating cell growth and cell death in a human teratocarcinoma derived cell line. *Int J Dev Biol* 1995;39:759-64.
- 11 Granger M, Engström W. Growth factors and apoptosis. *Cell Prolif* 1996;29:309-14.
- 12 Reynolds ML, Ward A, Graham CF, et al. Decreased skin sensory innervation in transgenic mice overexpressing insulin like growth factor II. *Neuroscience* 1997;79:789-97.
- 13 Talts JF, Aufderheide E, Sorokin L, et al. Induction of mouse tenascin expression by a human sarcomatoid Wilms tumour cell line growing in nude mice. *Int J Cancer* 1993;54:868-74.
- 14 Hyldahl L, Schofield PN, Engström W. Stimulatory effects of basic fibroblast growth factor on DNA synthesis in the human embryonic cornea. *Development* 1990;109:605-11.
- 15 Haanen C, Steffens-Nakken H, Reutelingsperger CPM. A novel assay for apoptosis. Flow cytometric detection of phosphatidyl expression on early apoptotic cells using fluorescein labelled annexin V. *J Immunol Methods* 1995;184:39-51.
- 16 Shima N, Itaki Y, Nagao M, et al. A fibroblast derived tumor cytotoxic factor—F-CCCTF—(hepatocyte growth factor/HGF) has multiple functions in vitro. *Cell Biol Int Rep* 1991;15:397-405.
- 17 Glucksman PD, Ambler GR. What is the function of insulin like growth factor II in postnatal life? *Mol Cell Endocrinol* 1993;92:C1-3.
- 18 Biddle C, Li CH, Schofield PN, et al. Insulin like growth factors and the multiplication of Tera 2—a human teratoma derived cell line. *J Cell Sci* 1988;90:475-85.
- 19 Ward A, Bierke P, Pettersson E, et al. Insulin like growth factors—growth, transgenes and imprinting. *Zool Sci* 1994;11:167-74.
- 20 Hill D. Relative abundance and molecular size of insulin like growth factors I and II in human fetal tissues. *Early Hum Dev* 1990;21:49-58.
- 21 Brice AL, Cheetham JE, Bolton VN, et al. Temporal changes in expression of the insulin like growth factor II gene associated with tissue maturation in the human fetus. *Development* 1989;106:543-54.
- 22 Schofield PN. Developmental tumours. *Br Med Bull* 1991;47:227-43.
- 23 Schofield PN, Lee A, Hill DJ, et al. Tumour suppression associated with expression of human insulin like growth factor II. *Br J Cancer* 1991;63:687-92.
- 24 Schofield PN, Granger M, Tally M, et al. The biological effects of a high molecular weight form of IGF II in a pluripotent human teratocarcinoma cell line. *Anticancer Res* 1994;14:533-8.
- 25 Reeve AH, Shih SA, Raizis AM, et al. Loss of allelic heterozygosity at a second locus on chromosome 11 in sporadic Wilms tumour cells. *Mol Cell Biol* 1989;9:1799-803.
- 26 Koufos A, Hansen MF, Copeland NE, et al. Loss of heterozygosity in three embryonic tumours suggests a common pathogenetic mechanism. *Nature* 1985;316:330-4.
- 27 Oda H, Shimizu Y, Inoue T, et al. Loss of imprinting of the IGF II gene in a Wilms tumour in an adult. *J Natl Cancer Inst* 1985;89:1813-14.
- 28 Haselbacher GK, Irminger JC, Zapf J, et al. Insulin like growth factor in human adrenal pheochromocytomas and Wilms tumours. Expression of the mRNA and protein level. *Proc Natl Acad Sci U S A* 1987;84:1104-6.



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