

# An Insulin-like Growth Factor-mediated, Phosphatidylinositol 3' Kinase-independent Survival Signaling Pathway in $\beta$ Tumor Cells<sup>1</sup>

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## Abstract

Hyperproliferation of tumor cells usually coincides with increased tumor cell apoptosis. To overcome apoptosis, tumor cells frequently induce the expression of growth factors that mediate cell survival. In nontransformed cells, including fibroblasts and neurons, survival factor-mediated signal transduction involves the activation of phosphatidylinositol 3' kinase (PI-3K) and protein kinase B/c-Akt (PKB). Here we demonstrate that tumor cell lines derived from a transgenic mouse model of pancreatic  $\beta$  cell carcinogenesis use insulin-like growth factors to repress apoptosis independently of PI-3K and PKB. The results indicate that tumor cells can use additional survival signal transduction pathways.

## Introduction

The balance between tumor cell proliferation and tumor cell apoptosis is a critical determinant of malignant tumor outgrowth. To prevent programmed cell death, tumor cells frequently evolve to express secreted growth factors that induce survival signaling pathways, thereby repressing apoptosis. For example, up-regulation of either the IGF-1<sup>3</sup> receptor or its ligands IGF-I and IGF-II in an autocrine fashion is observed in a large number of tumor types (1–2). Recent experiments with tumor cell lines *in vitro* (1–3) and with a transgenic mouse model of  $\beta$  cell carcinogenesis *in vivo* (4) clearly indicated that IGFs act as survival factors to suppress tumor cell apoptosis. Transformation of cells and their need to overcome apoptosis is nicely exemplified in cultured fibroblasts that have been transfected, for example, by the proto-oncogene *c-myc*. Expression of *c-myc* readily induces apoptosis on serum-deprivation; the addition of IGF-I or platelet-derived growth factor (5) or forced expression of *Bcl-2* (6) repress apoptosis of these cells. Similarly, primary cultures of neurons also rapidly undergo apoptosis when serum-deprived, and IGFs and a number of neurotrophic growth factors are able to prevent this apoptosis (7). Experiments with fibroblasts and neurons have been instrumental in deciphering the signaling pathways that are used by IGFs and their cognate receptor, the IGF-1R, to suppress apoptosis. Survival signal transduction by the IGF-1R has been shown to involve the activation of PI-3K and PKB (8–10). On stimulation of the IGF-1R and phosphorylation of IRS-1, the p85 regulatory subunit of PI-3K associates with phosphotyrosine residues on IRS-1. Subsequently, PI-3K associates with membranes where the p110 catalytic subunit of PI-3K phosphorylates phosphoinositides (11). The resulting phosphorylated phosphoinositides, mainly phosphatidylinositol-3,4-

phosphate (PtdIns-3,4-P<sub>2</sub>), trigger the phosphorylation and activation of PKB by upstream phosphatidylinositol-phosphate-dependent kinases (PDK-1 and PDK-2; Ref. 12). One of the several downstream substrates of PKB is Bad, an apoptosis-inducing member of the Bcl-2 family of proteins (13). Bad, on phosphorylation by PKB, is sequestered by 14-3-3 proteins, thereby losing its ability to bind and inactivate Bcl-2 and Bcl-x<sub>L</sub>, apoptosis-inhibiting members of the Bcl-2 family. Bcl-2 and Bcl-x<sub>L</sub> are then free to suppress the execution of apoptosis. However, although this pathway has been repeatedly demonstrated in nontransformed cell types, it has remained elusive whether in tumor cells IGF-mediated survival uses the same signal transduction pathway.

## Materials and Methods

Plasmids encoding adenoviral gene products E1A and E1B-19K have been described previously (3). The plasmid encoding EGFP (pEGFP-C1) was purchased from Clontech. The dominant-negative IGF-1R construct was described previously (3). The plasmid encoding a dominant-negative version of FGF receptor-1 was a gift from Dr. S. Werner (Max-Planck-Institute for Biochemistry, Munich, Germany; Ref. 14). Plasmids encoding PKB and v-PKB were a gift from Dr. M. Olt (IMP, Vienna; Ref. 15). Human recombinant IGF-II (Calbiochem), human recombinant EGF (Promega), staurosporine (Sigma), wortmannin (Sigma), LY294002 (Calbiochem), okadaic acid (Sigma), rapamycin (Calbiochem), calyculin A (Life Technologies, Inc.), tautomycin (Calbiochem), Et180CH3 (Calbiochem), PD98059 (Calbiochem), and anisomycin (Calbiochem) were resuspended and stored according to the suppliers' recommendations.

Establishment and maintenance of  $\beta$ TC cell lines, either wild-type ( $\beta$ TC: IGF-II+/+) or deficient ( $\beta$ TC:IGF-II–/–) for IGF-II expression, have been described previously (3). The apoptotic index was determined by propidium iodide staining and subsequent flow cytometric analysis of the cellular DNA content. The incidence of apoptosis is plotted in the bar graphs as the percentage of cells that have a DNA content of less than 2 N (sub-2 N). For each measurement, 10,000 events gated for size and single cells were collected. Transient cotransfection of  $\beta$  tumor cells with plasmids encoding EGFP and genes of interest and the subsequent analysis of the rate of apoptosis by flow cytometry has been described previously (16).

The specific activity of PI-3K was determined as described previously (17). Briefly,  $\beta$  tumor cell lines were serum-deprived for 24 h, then treated with 200 nM wortmannin 5 h before lysis. Ten min before lysis, cells were stimulated with 1.5  $\mu$ g/ml insulin. Medium was removed, cells were washed three times with wash buffer A [137 mM NaCl, 20 mM Tris-HCl (pH 7.4), 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 100  $\mu$ M sodium orthovanadate] and then lysed in 500  $\mu$ l of lysis buffer (wash buffer A containing 1% NP40, 10  $\mu$ g/ml aprotinin, 10  $\mu$ g/ml leupeptin, and 100  $\mu$ M PMSF). PI-3K complexed to IRS-1 was immunoprecipitated with 1.66  $\mu$ g of polyclonal anti-IRS-1 antibody (Upstate Technologies) as described previously (17). The pellet was washed three times with lysis buffer, three times with wash buffer B [0.1 M Tris-HCl (pH 7.4), 5 mM LiCl<sub>2</sub>, and 100 mM sodium orthovanadate] and twice with TNE [10 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 150 mM NaCl]. Specific PI-3K activity was determined by resuspending the pellet in 40  $\mu$ l of TNE and the addition of 20  $\mu$ l of phosphoinositol [2 mg/ml in 10 mM Tris-HCl (pH 7.4), and 1 mM EGTA], 10  $\mu$ l of 100 mM CaCl<sub>2</sub>, and 5  $\mu$ l of ATP working-solution (880  $\mu$ M ATP containing 400  $\mu$ Ci/ml [ $\gamma$ -<sup>32</sup>P]ATP, 20 mM MgCl<sub>2</sub>) for 10 min. at 37°C. The reaction was terminated by the addition of 20  $\mu$ l of 6 N HCl; labeled lipid was

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<sup>3</sup> The abbreviations used are: IGF, insulin-like growth factor; PI-3K, phosphatidylinositol 3' kinase; PKB, protein kinase B/c-Akt; IRS-1, insulin receptor substrate 1; EGFP, enhanced green fluorescent protein; IGF-1R, IGF-1 receptor; MBP, myelin basic protein; c-PKB, wild-type PKB; v-PKB, constitutive-active form of PKB; dnPKB, dominant-negative form of PKB; FGF, fibroblast growth factor; EGF, epidermal growth factor.

extracted with 160  $\mu$ l  $\text{CHCl}_3$ /methanol (1:1); and 50  $\mu$ l of the chloroform phase were analyzed by TLC on a TLC-plate (Sigma). The TLC-plate was developed in a saturated chamber with running buffer [ $\text{CHCl}_3$ /methanol/ $\text{H}_2\text{O}$ / $\text{NH}_3$  (60:47:11:3:2)] and exposed to a phosphorimager screen. Labeled lipids were quantitated using Imagequant software.

The specific activity of PKB was determined as described previously (15). Briefly, cells were serum-deprived for 24 h, and 5 h before harvesting, the cells were treated with wortmannin (200 nM). Cells were lysed in 500  $\mu$ l lysis buffer [20 mM Tris-HCl (pH 7.5), 40 mM sodium pyrophosphate, 50 mM NaF, 5 mM  $\text{MgCl}_2$ , 100  $\mu$ M sodium vanadate, 10 mM EDTA, 1% Triton X-100, 0.5% sodium deoxalate, 0.1% SDS, 20  $\mu$ g/ml aprotinin and leupeptin, and 3 mM PNP]. PKB was immunoprecipitated with 2  $\mu$ g of polyclonal anti-PKB antibody (New England Biolabs). The pellet was washed three times with lysis buffer and three times with wash buffer [50 mM Tris-HCl (pH 7.5), 10 mM  $\text{MgCl}_2$ ]. Half of the pellet was used for the quantitation of PKB protein by immunoblotting analysis. The other half was resuspended in 25  $\mu$ l of assay buffer [50 mM Tris-HCl (pH 7.5), 10 mM  $\text{MgCl}_2$ , 1 mM DTT, 50  $\mu$ M ATP, 400  $\mu$ g/ml MBP (Sigma), and 120  $\mu$ Ci/ml [ $\gamma$ - $^{32}\text{P}$ ]ATP] and incubated at room temperature for 15 min. The reaction was terminated by boiling in SDS sample solution, and labeled MBP was visualized and quantitated by SDS-polyacrylamide gel electrophoresis and phosphorimager analysis using Imagequant software. Relative PKB activity was calculated by normalizing the enzymatic activity to the amount of protein present in the immunoprecipitate.

## Results and Discussion

Using a transgenic mouse model of  $\beta$ -cell carcinogenesis (Rip1Tag2) we have recently demonstrated that IGF-II acts as a survival factor during tumorigenesis *in vivo* (4). In this transgenic mouse model, expression of IGF-II is up-regulated concomitant with the onset of tumor cell hyperproliferation. On crossing the Rip1Tag2 transgenic mice with IGF-II knockout mice, tumor outgrowth is dramatically reduced and this can be accounted for by an increase in tumor cell apoptosis. This survival function of IGF-II is recapitulated in  $\beta$  tumor cell lines derived from IGF-II-deficient Rip1Tag2 mice ( $\beta\text{TC:IGF-II}^{-/-}$ ; Ref. 3), which exhibited a higher incidence of apoptosis as compared with their wild-type counterparts ( $\beta\text{TC:IGF-II}^{+/+}$ ).

For example,  $\beta\text{TC:IGF-II}^{-/-}$  are more sensitive to apoptotic stim-

uli, such as serum deprivation, staurosporine (Fig. 1A), and okadaic acid (Fig. 1B), and to chemotherapeutic agents, such as daunomycin, etoposide, or vincristine (3). Notably, the lack of IGF-mediated survival function potentiates the killing of  $\beta$  tumor cells by various chemotherapeutic agents (3). IGF-II-deficient  $\beta$  tumor cells ( $\beta\text{TC:IGF-II}^{-/-}$ ) can be rescued from apoptosis by the addition of recombinant IGF-I or IGF-II but not by EGF (Fig. 1B; Ref. 3) or other growth factors tested (data not shown).  $\beta\text{TC:IGF-II}^{+/+}$  cells are also sensitized to chemotherapy when transfected with a dominant-negative version of the IGF-1R, to an extent that is comparable with adenovirus 5 *E1A* gene product, an inducer of apoptosis (Fig. 1C). In contrast, a dominant-negative mutant FGF receptor does not affect apoptosis of  $\beta$  tumor cells (Fig. 1C). Together, the data demonstrate that  $\beta$  tumor cell lines use IGF-II and the activated IGF-1R as a specific survival signal to protect against a variety of apoptotic stimuli.

We used several independent  $\beta\text{TC:IGF-II}^{+/+}$  and  $\beta\text{TC:IGF-II}^{-/-}$  cell lines to characterize the signaling pathway by which IGFs repress the execution of apoptosis and, thus, allow increased tumor cell survival. Similar to fibroblasts and neurons (5, 7), IGF-mediated survival function of  $\beta$  tumor cell lines may require the activation of PI-3K, and, hence, we treated  $\beta\text{TC:IGF-II}^{+/+}$  and  $\beta\text{TC:IGF-II}^{-/-}$  with two specific inhibitors of PI-3K activity, wortmannin and LY294002, respectively. If PI-3K would be required for IGF-mediated survival of  $\beta$  tumor cells, treatment with these compounds should induce significant apoptosis of  $\beta\text{TC:IGF-II}^{+/+}$  but not of  $\beta\text{TC:IGF-II}^{-/-}$  cells. Unexpectedly, however, the addition of wortmannin (Fig. 2A) or LY294002 (data not shown) in concentrations that completely inhibited PI-3K activity (Fig. 2B; data not shown) did not reveal any significant increase of apoptosis in  $\beta\text{TC:IGF-II}^{+/+}$ . Due to their lack of IGF-II expression,  $\beta\text{TC:IGF-II}^{-/-}$  exhibited a higher background level of apoptosis, but the incidence of apoptosis was also not further increased by the PI-3K inhibitors in these cells (Fig. 2A). To ensure that the addition of the inhibitors resulted in efficient repression of PI-3K activity, PI-3K was immunoprecipitated from the different cell lines, and the specific PI-3K activity was determined.

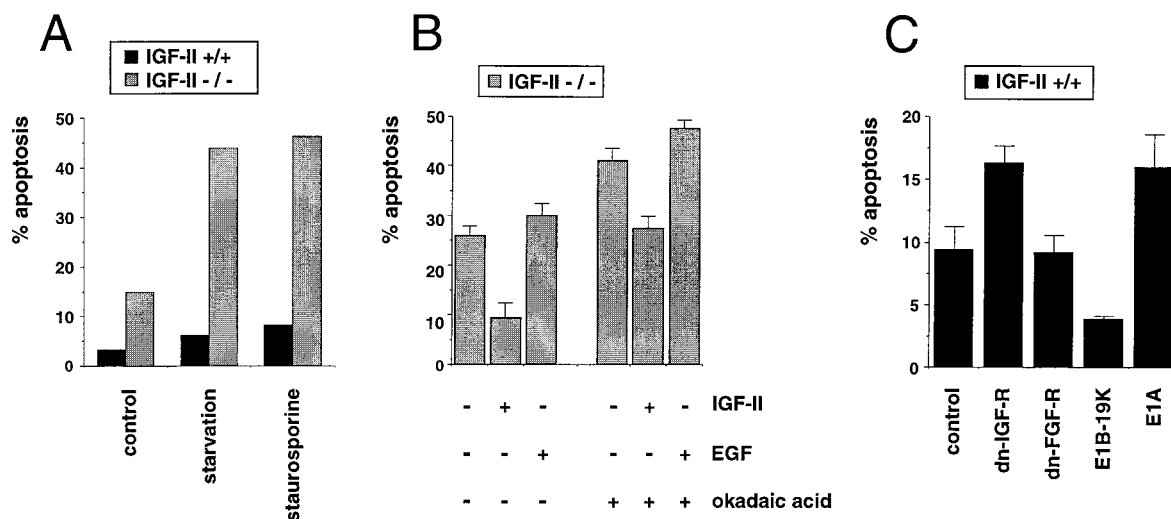


Fig. 1. IGF-II acts as a survival factor for  $\beta$  tumor cells via the IGF-1R. A, IGF-II-deficient tumor cells ( $\text{IGF-II}^{-/-}$ ; gray columns) show a higher incidence of apoptosis than their wild-type counterparts ( $\text{IGF-II}^{+/+}$ ; black columns) even under high-serum conditions (control; 10% FCS) and are more susceptible to serum deprivation (starvation; 0% FCS), and staurosporine treatment (400 ng/ml). B, IGF-II but not EGF is able to suppress serum deprivation- and okadaic acid-induced apoptosis in IGF-II-deficient tumor cells ( $\text{IGF-II}^{-/-}$ ), which indicates that the lack of survival factor function is responsible for increased apoptosis in IGF-II-deficient  $\beta$  tumor cells. IGF-II-deficient tumor cells were grown in reduced serum (5% FCS) in the absence or in the presence of okadaic acid (100 nM). Recombinant IGF-II (50 ng/ml) or EGF (10 ng/ml) was added as indicated. C, expression of a dominant-negative IGF-1R in wild-type  $\beta$  tumor cells results in increased apoptosis at levels that are comparable with the expression of the adenovirus 5 *E1A* gene product, an inducer of apoptosis. IGF-II<sup>+/+</sup> cells were cotransfected with a plasmid encoding EGFP together with either a control plasmid (control) or expression plasmids encoding adenovirus *E1A* (*E1A*), adenovirus *E1B-19K* (*E1B-19K*), dominant-negative FGF receptor (*dnFGF-R*), or dominant-negative IGF-1R (*dnIGF-1R*). Apoptotic cells were quantitated as described in "Material and Methods." Error bars, SD.

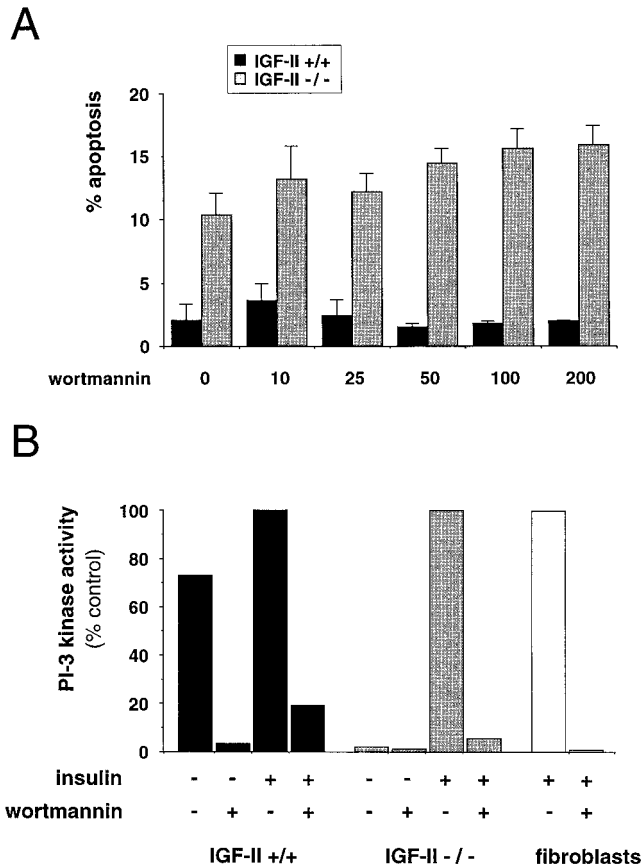


Fig. 2. IGF-II-mediated survival function is PI-3K-independent. A, wortmannin, a potent inhibitor of PI-3K activity, does not significantly affect the rate of apoptosis in wild-type  $\beta$  tumor cells (IGF-II<sup>+/+</sup>; black columns) and IGF-II-deficient  $\beta$  tumor cells (IGF-II<sup>-/-</sup>; gray columns). Notably, the levels of apoptosis in wild-type  $\beta$  tumor cells are not increased to the levels of apoptosis that are found in the absence of IGF-II, which indicates that PI-3K is not involved in the execution of IGF-mediated survival function. Cells were cultured under high-serum conditions for 48 h in the presence of increasing concentrations of wortmannin (0–200 nM). Apoptotic cells were quantitated as described in “Material and Methods.” Error bars, SD. B, whereas wortmannin does not affect  $\beta$  tumor cell apoptosis, it inhibits PI-3K activity in  $\beta$  tumor cells. Wild-type (IGF-II<sup>+/+</sup>; black columns) and IGF-II-deficient (IGF-II<sup>-/-</sup>; gray columns)  $\beta$  tumor cells were treated with insulin (1.5  $\mu$ g/ml) and wortmannin (200 nM) as indicated. Specific PI-3K activity was determined by an immunoprecipitation/kinase assay as described in “Material and Methods.” Primary mouse fibroblasts (open columns) served as control for insulin-induced PI-3K activity. Maximal stimulation by insulin within a particular cell type was set to 100% PI-3K activity. In contrast to IGF-II-deficient  $\beta$  tumor cells, wild-type  $\beta$  tumor cells exhibit high PI-3K activity in the absence of insulin, most likely induced by the presence of IGF-II. This activity as well as the insulin-induced activity is efficiently repressed by wortmannin.

Stimulation of PI-3K by treatment of the cells with insulin was used as a positive control. These experiments reveal that PI-3K activity is almost as high in the presence of IGF-II as compared with stimulation with insulin. By contrast, PI-3K is absent in IGF-II-deficient  $\beta$  tumor cells under normal growth conditions but is readily stimulated to high levels by insulin (Fig. 2B). In both IGF-II<sup>+/+</sup> and IGF-II<sup>-/-</sup>  $\beta$  tumor cell lines, PI-3K activity is completely repressed by the addition of wortmannin (Fig. 2B) or LY294002 (data not shown). Together, the results indicate that IGF-II via the IGF-1R is able to induce PI-3K activity. However, in contrast to the reported effects of IGFs on fibroblasts and neurons (12,13, 18, 19), activation of PI-3K does not appear to be solely responsible for IGF-II/IGF-1R-mediated survival signaling in  $\beta$  tumor cells.

Because PKB has been reported to be required for survival signaling in nontransformed cells (12, 13, 18, 19), we next examined whether IGF-mediated survival function in  $\beta$  tumor cells involved the activation of PKB in an PI-3K-dependent or independent manner. Apoptosis of IGF-wild-type and IGF-II-deficient  $\beta$  tumor cell lines

was stimulated by serum deprivation concomitant with the treatment of the cells with wortmannin, and subsequently PKB activity was determined by a specific immunoprecipitation/kinase assay (Fig. 3A). PKB activity in both  $\beta$ TC:IGF-II<sup>+/+</sup> and  $\beta$ TC:IGF-II<sup>-/-</sup> was significantly affected by neither changes in PI-3K activity (treatment with wortmannin) nor changes in the extent of apoptosis (compare  $\beta$ TC:IGF-II<sup>-/-</sup> with and without serum deprivation). LY294002, another potent inhibitor of PI-3K, also did not affect IGF-mediated survival activity (data not shown). Consistent with these results, transient transfections of  $\beta$ TC:IGF-II<sup>-/-</sup> with cDNA constructs encoding c-PKB, v-PKB, or dnPKB did not significantly affect the extent of tumor cell apoptosis, whereas the adenovirus 5 *E1A* and *E1B 19K* gene products, well-known inducer and inhibitor of apoptosis, respectively, were able to modulate the levels of apoptosis in these experiments (Fig. 3B). Together, the results indicate that the activation of PKB is not required for IGF-mediated survival function in  $\beta$  tumor cell lines.

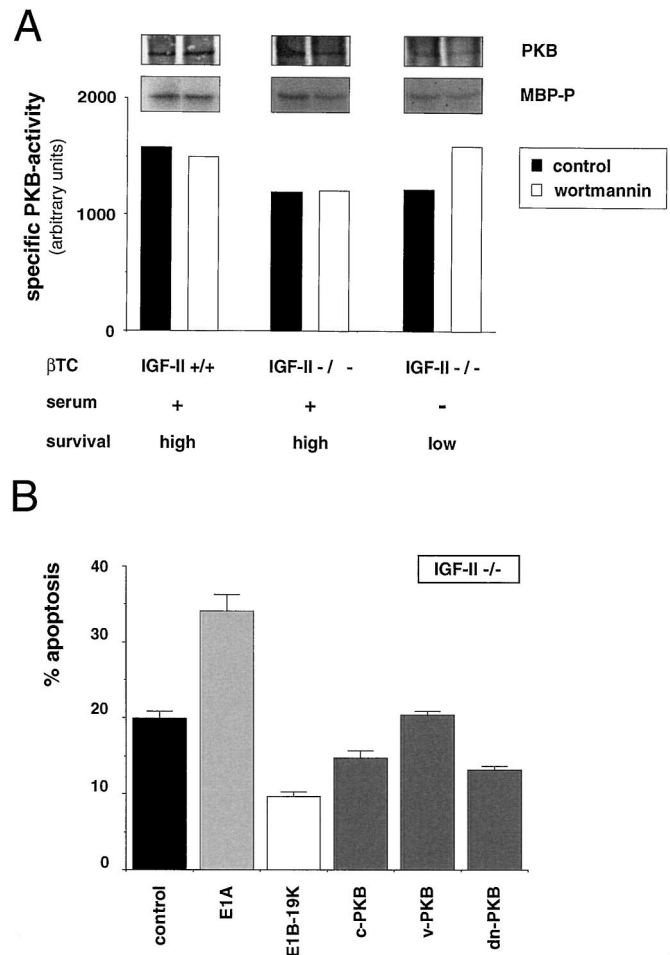


Fig. 3. IGF-II-mediated survival function is independent of the activity of PKB. A, wortmannin, a potent inhibitor of PI-3K activity, does not affect PKB activity. Wild-type (IGF-II<sup>+/+</sup>) and IGF-II-deficient (IGF-II<sup>-/-</sup>)  $\beta$  tumor cells were grown in the presence (serum +) or absence of serum (serum -) and in the absence (black columns) or in the presence of wortmannin (200 nM; open columns) as indicated. PKB activity was not affected by differences in IGF-II expression and tumor cell survival as indicated at the bottom. PKB was immunoprecipitated, PKB protein levels were quantitated by immunoblotting (upper part of the panel, PKB), and PKB activity was determined using MBP as a substrate (below; MBP-P) as described in “Material and Methods.” Bar graph, specific PKB activities, calculated as the ratio of PKB protein levels:PKB activities. B, modulation of PKB activity by transient transfection does not affect apoptosis of IGF-II-deficient  $\beta$  tumor cells (IGF-II<sup>-/-</sup>). Cells were cotransfected with a plasmid encoding EGFP together with either a control plasmid (control) or expression plasmids encoding adenovirus *E1A* (*E1A*), adenovirus *E1B-19K* (*E1B-19K*), wild-type PKB (*c-PKB*), constitutive-active PKB (*v-PKB*), or a dominant-negative form of PKB (*dnPKB*). Apoptotic cells were quantitated as described in “Material and Methods.” Error bars, SD.



The most prominent substrate of IGF-1R that is known to transmit ligand binding to downstream effectors is IRS-1 (1, 2). Immunoprecipitation and immunoblotting experiments with antibodies against IRS-1 did not reveal any significant changes in the protein levels of IRS-1 nor in the extent of phosphorylation of IRS-1 between  $\beta$ TC: IGF-II+/+ and  $\beta$ TC:IGF-II-/- cells (data not shown). Hence, the data suggest that in  $\beta$  tumor cells, IGF-mediated survival signal transduction is not using the well-documented pathway involving IRS-1, PI-3K, and PKB. Rather, other signaling cascades may be involved. To test for this possibility, we treated wild-type and IGF-II-deficient  $\beta$  tumor cell lines with a number of known inhibitors of other signal transduction pathways. In particular, in these experiments, we screened for substances that specifically interfered with IGF-mediated survival function, *i.e.*, substances that induced apoptosis in wild-type  $\beta$  tumor cell lines but did not change the levels of apoptosis in IGF-II-deficient  $\beta$  tumor cell lines. Notably, of the large number of compounds tested, none exhibited IGF-specific inhibition of tumor cell survival (Table 1). Transfection of cDNA constructs encoding activated forms of the proto-oncogenes *c-Raf* and *c-Ras* also did not affect IGF-mediated survival function (data not shown).

Similar to most tyrosine kinase receptors, the IGF-1R uses a variety of substrates that funnel into different signaling pathways. In part, these pathways represent the different biological mechanisms that are modulated by IGFs, such as mitogenicity, transformation, and the inhibition of apoptosis. Notably, mutational analysis of the IGF-1R revealed that distinct domains in the cytoplasmic parts of the receptor mediated the different functions (18–20). In a simplified way, IGF-1R-specific signaling may be organized in two major arms of signal transduction: the proliferation pathway (MAP kinase pathway) and the anti-apoptotic pathway (PI-3K pathway). However, a clear separation of these pathways is not possible; the two pathways appear to interact with each other and to a certain extent may even be able to replace each other. Notably, Ras is not only a major transducer of the mitogenic signals conveyed by the IGF-1R but also seems to act upstream of the activation of PI-3K (8). Such an interplay between the PI-3K pathway and the MAP kinase pathway in survival function was also demonstrated in PC12 cell lines in which serum deprivation-induced apoptosis was suppressed by IGF-I (21).

Our results seem to exclude these well-characterized pathways from the IGF-mediated repression of apoptosis in  $\beta$  tumor cells. Could alternate IGF-mediated survival signaling pathways be tumor cell-specific? Recently, Kulik and Weber (22) have reported that overexpression of the IGF-1R in fibroblasts resulted in the engagement of an

IGF-1R-mediated survival signal that exerts its function independently of PI-3K and PKB activities. Moreover, Soon *et al.* (23) have shown that MAP kinase but not PI-3K and IRS-1 is required for IGF-mediated hematopoietic cell proliferation. Similar to the  $\beta$  tumor cell lines used in our experiments, expression of IGF-ligands or of the IGF-1R is frequently up-regulated in many cancer cells (1, 2), arguing for a tumor cell-specific induction of an alternate signaling pathway. Together, the data indicate that novel anti-apoptotic signaling pathways are still to be elucidated, and that PI-3K or PKB may not be the only potential targets for therapeutic approaches that are based on the inhibition of tumor cell survival signals.

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Table 1 Inhibition of IGF-mediated survival function

Compound <sup>a</sup>	Target activity	Nonspecific <sup>b</sup>	IGF-specific <sup>c</sup>
Wortmannin (1 $\mu$ M)	Inhibitor of PI-3K	—	—
LY294002 (33 $\mu$ M)	Inhibitor of PI-3K	—	—
Rapamycin (200 ng/ml)	Inhibitor of p70 S6 kinase	—	—
Okadaic acid (1 $\mu$ M)	Inhibitor of PP1 <sup>d</sup> , less PP2A <sup>d</sup>	+	—
Calyculin A (200 nM)	Inhibitor of PP1 and PP2A	—	—
Tautomycin (1 $\mu$ M)	Inhibitor of PP2A, less PP1	—	—
Et180CH3 (300 $\mu$ M)	Inhibitor of phospholipase C	+	—
Staurosporine (1 $\mu$ M)	Inhibitor of protein kinase C	+	—
PD98059 (300 $\mu$ M)	Inhibitor of MAP kinase	—	—
Anisomycin (20 $\mu$ g/ml)	Activator of MAP kinase	+	—

<sup>a</sup> Compounds were titrated over a wide range in these experiments; maximum concentrations are given in parentheses.

<sup>b</sup> Reagents that induced apoptosis of both IGF-II wild-type and IGF-II-deficient  $\beta$  tumor cell lines were considered nonspecific inhibitors; they are marked by +. The rate of apoptosis of IGF-II+/+ and IGF-II-/-  $\beta$  tumor cell lines was determined as described in "Material and Methods."

<sup>c</sup> Reagents that exclusively induced apoptosis of IGF-II wild-type  $\beta$  tumor cell lines were considered specific inhibitors of IGF-mediated survival function. None of the reagents tested fulfilled this criterion.

<sup>d</sup> PP, protein phosphatase 1; PP2A, protein phosphatase 2A.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## An Insulin-like Growth Factor-mediated, Phosphatidylinositol 3' Kinase-independent Survival Signaling Pathway in $\beta$ Tumor Cells

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