Insulin-like Growth Factor 1 Receptor Enhances Invasion and Induces Resistance to Apoptosis of Colon Cancer Cells through the Akt/Bcl-xL Pathway

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ABSTRACT

Colon cancer overexpresses insulin-like growth factor 1 (IGF1) and insulin-like growth factor 1 receptor (IGF1-R), as compared with normal or adenomatous mucosa, and it has been postulated that colorectal cancer cells depend on the IGF1/IGF1-R pathway for their growth and progression. In this study, using the human colon cancer cell line HCT116, we find that established HCT116/IGF1-R transfectants exhibit a more aggressive transformed phenotype than the parental cell line, as demonstrated by their higher proliferation rate in response to IGF1, higher degree of anchorage-independent growth, resistance to serum deprivation-induced apoptosis, and higher migratory capability in a monolayer “wounding assay.” When injected into nude mice, HCT116/IGF1-R transfectants were highly invasive and produced distant metastases, whereas the parental cell did not. Moreover, the overexpression of IGF1-R in these cells was associated with IGF1-R-induced activation of Akt and up-regulation of the antiapoptotic protein Bcl-xL. We also show that Akt pathway mediates IGF1-R-induced Bcl-xL expression at transcriptional level. Our data demonstrate, for the first time, that IGF1-R/Akt/Bcl-xL pathway may contribute to a more aggressive malignant phenotype, in a subset of colorectal cancers.

INTRODUCTION

Colorectal adenocarcinoma is one of the most common malignancies, accounting for ~15% of all cancer-related deaths in United States (1). If not diagnosed and treated early, this tumor spreads to the entire bowel wall, extends to adjacent organs, and eventually metastasizes to regional lymph nodes and distant sites. The majority of deaths from colorectal cancer occurs in patients with late stage tumors, which are usually incurable (2).

Well-defined molecular alterations have been associated with colorectal cancer progression including adenomatous polyposis coli, ras gene mutations, and alterations of DCC, DPC-4, p53, and c-src genes (3–7). These changes in gene expression are thought to trigger multiple downstream signals, thereby providing tumor cells with new malignant attributes.

It is now evident that the aberrant expression of IGF1-R is an additional molecular alteration occurring during colorectal cancer progression (8–14). IGF1-R is a heterotetramer with tyrosine kinase activity and is composed of two extracellular α subunits containing the ligand-binding site and two transmembrane β subunits harboring the intracellular tyrosine kinase activity, connected by disulfide bonds (15–19). Knockout studies in mouse have shown that IGF1 and IGF1-R are key regulators of cell growth and development (20), and that they are required for cell cycle progression (21, 22). When activated by the binding of specific ligands (IGF1 and IGF2), IGF1-R induces a cascade of intracellular tyrosine phosphorylations that eventually culminate in the activation of transcription factors playing a role in transformation (22–24), tumor invasion (25), metastasis (26), and protection from apoptosis (27–29). There is now an increasing body of evidence that a subset of colorectal cancers may be under autocrine regulation of the IGF1/IGF1-R system (8–14), and that a functional IGF1-R may have a key role in the survival of transformed colonocytes by inhibiting apoptosis (30). It seems that the overexpression of IGF1-R confers to cells resistance to apoptosis under a wide variety of conditions including growth factor withdrawal (31), serum deprivation (32), incubation with tumor necrosis factor (30), activation of interleukin 1β-converting enzyme-like proteases (33), and UV B irradiation (34). Conversely, numerous studies have shown that tumor cells can be driven into apoptosis using IGF1-R blocking strategies including the use of antisense technology, dominant negative mutants, or triple helix formation. All of these strategies have led to decreased tumorigenesis and metastatic potential, mainly by inducing apoptosis of cancer cells (35–38). It has been postulated that the antiapoptotic function of IGF1-R depends on its ability to inhibit interleukin 1β-converting enzyme-like caspases and to increase the activity and expression of the negative death regulator Bcl-xL (33, 39).

We hypothesize that changes in the expression of IGF1-R may trigger signals activating molecular pathways linked to transformation, tumorigenesis, metastasis, and cell survival, ultimately inducing modifications in the behavior of colon cancer cells. To test this hypothesis, we transfected a human colon cancer cell line exhibiting minimal levels of IGF1-R (HCT116) with an expression plasmid encoding the full length protein sequence of the human IGF1-R, to determine the contributions of IGF1-R to the HCT116/IGF1-R phenotype as compared with that of the parental cell line (HCT116). Our data indicate that the presence of an overexpressed and functional IGF1-R triggers a variety of signals inducing a higher degree of proliferation and transformation, resistance to serum deprivation-induced apoptosis, and increased migratory capabilities of colon cancer cells. Significantly, we found that HCT116/IGF1-R transfectants, when injected s.c. into nude mice, produced larger tumors that were highly invasive and metastasizing, as compared with the parental cell line. Furthermore, we observed that the antiapoptotic function of IGF1-R in HCT116/IGF1-R transfectants was associated with activation of Akt and up-regulation of Bcl-xL proteins.

MATERIALS AND METHODS

Cell Lines and Cell Culture. The human colon carcinoma cell line HCT116 was obtained from the American Type Culture Collection (Manassas, VA). The cells were maintained under standard culture conditions in RPMI 1640 (Whittaker MA Bioproducts, Walkersville, MD) supplemented with 10% FBS (HyClone Laboratories, Logan, UT).

Plasmid Transfection. HCT116 cells were seeded in 60-mm Petri dishes at a density of 0.5 × 106 cells/dish. After overnight incubation, the cells were
transfected with 2 μg of DNA per dish, using LipofectAMINE Plus (Life Technologies, Inc.). The expression construct included a CVN expression vector containing the full-length coding sequence of the human IGF1-R-cDNA and the neomycin resistance gene, both under the control of the SV40 early promoter. This plasmid was kindly provided by Dr. R. Baserga (Jefferson Cancer Center, Philadelphia, PA). After 48 h, 0.6 mg/ml G418 was added to the cells, and three stable clonal cell lines were established (HCT116/IGF1-R).

**Surface IGF1-R Expression.** Determination of the cell surface IGF1-R levels on HCT 116 and HCT116/IGF1-R clonal cells was performed by flow cytometry. Exponentially growing cells (HCT116 and HCT116/IGF1-R clones) were trypsinized and washed twice with PBS, centrifuged, resuspended, and counted. The cells at a concentration of 10^6 cells/sample in FACS buffer were incubated for 30 min on ice with 5 μg/ml IGF1-R-phycoerythrin-conjugated mouse IgG1 antibody (BD PharMingen, San Diego, CA). Background staining was performed with a mouse IgG1 isotype control. Cells were washed and resuspended in 0.5 ml of FACS buffer. They were analyzed for fluorescence intensity on a FACSCaliber using CellQuest software (BD Biosciences, San Jose, CA).

**Western Blot Analysis.** After culture for 24 h in 10% serum-enriched medium, HCT116 and/or HCT116/CVN and HCT116/IGF1-R clonal cells were subjected to Western blot analysis as previously described (23). The primary antibodies used for the Western blot included: rabbit antihuman polyclonal IGF1-R antibody to cross-react with the ~85 kDa β subunit of the IGF1-R (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), fluorescence-activated cell sorter and phospho-Akt antibodies (Cell Signaling Technology, Inc.), mouse antihuman Bcl-xL antibodies (ND PharMingen), and monoclonal anti-β-actin antibody (Sigma-Aldrich, St. Louis, MO). The blots were washed and incubated with the appropriate HRP-conjugated secondary antibody (Oncogene Science, Inc., Uniondale, NY). Antigen bound to nitrocellulose membranes were detected with the ECL system (Amersham Corp., Arlington Heights, IL).

**IGF1-R Phosphorylation.** Subconfluent HCT116/CVN and HCT116/IGF1-R clonal cells were serum starved overnight and stimulated with or without 100 ng/ml IGF1 for 10 min at 37°C. Cells were washed twice in ice-cold PBS and lysed with 500 μl/plate lysis buffer [50 mM HEPES (pH 7.6), 150 mM sodium chloride, 1% Triton X-100, 2 mM sodium vanadate, 100 mM NaN3, and 0.40 mg/ml phenylmethylsulfonyl fluoride]. Equal amounts of proteins (500 μg) were immunoprecipitated with 1.0 μg of anti-IGF1-R antibody (Ab-1 from Oncogene Research Products, Beverly, MA) for 4 h at 4°C. The immunoprecipitates were incubated in the presence of protein A-G agarose, washed twice with lysis buffer, and subjected to Western blotting analysis. Protein tyrosine phosphorylation was detected by a 1:1000 dilution of HRP-conjugated antiphosphotyrosine monoclonal antibody (Upstate Biotechnology, Lake Placid, NY). Immunoblotting of IGF1-R β was performed using antihuman IGF1-R β HRP-conjugated polyclonal antibody (Santa Cruz Biotechnology). Detection of antigen-bound antibody was effected with the ECL Western blotting analysis System (Amersham).

**Promoter Activation Assays.** The murine bcl-x promoter-luciferase reporter plasmid was derived from a 3.2-kb genomic fragment containing the 5′-region of the bcl-x gene upstream of the ATG translational start codon and have been described in detail (40). HCT116/IGF1-R clonal cell lines and parental HCT116 cells were plated at a density of 5 x 10^4 cells/well in well-24 well tissue culture plates and, when adherent (24 h after plating), were transiently transfected using LipofectAMINE Plus (Life Technologies, Inc.), with a plasmid containing the luciferase gene driven by the bcl-x promoter, together with the human IGF1-R expression plasmid, or dominant negative Akt, or vector alone, and stimulated with or without IGF1 (100 ng/ml). After 36 h of transfection, cells were lysed and subjected to luciferase reporter assay.

**Cell Growth Assays.** The growth properties of HCT116 as compared with HCT116/IGF1-R (3 clones) and HCT116/CVN cells were assessed by [3H]thymidine incorporation in 2 x 10^4 cells/well in 96-well plates as follows. HCT116/CVN, and HCT116/IGF1-R cells were allowed to attach for
clonal cells as compared with the controls. Similarly, growth curve analysis showed a parental cells and the HCT116/CVN empty vector control, which did not respond. HCT116/IGF1-R clones to the added IGF1 was arrested medium with and without IGF1 (100 ng/ml) for 24 h. The DNA synthesis of significance.

cpm/well, with means ± SD of 3 experiments comprising 18 replicates. *, statistical significance.

24 h, incubated in growth arrest medium (0.1% FBS) for 24 h, and then incubated for an additional 24 h in fresh medium (0.1% FBS) containing IGF1 (100 ng/ml) for 24 h. For the last 6 h of incubation, 0.5 μCi/well [3H]thymidine (Perkin-Elmer Life Sciences, Boston, MA) was added. Cells were then harvested onto filters using a cell harvester and counted using a Beckman liquid scintillation counter. The results are expressed as cpm/well, with means ± SD of 3 experiments comprising 18 replicates. *, statistical significance.

Soft Agar Assay. To test the impact of IGF1-R expression on anchorage-independent colony formation, 1 × 103 cells (HCT 116, HCT116/CVN, and HCT116/IGF1-R clones) were suspended in complete medium containing 0.5% agar and plated in 6-well plates over a basal layer of complete medium containing 1% agar. Colonies >125 μm in diameter (or 10 cells) were counted at 14 and 21 days. The clonogenicity was determined in three independent experiments.

In Vitro Cell Motility Assay. In vitro cell motility assay was performed using the monolayer “wounding assay.” For this test, HCT 116 and HCT116/CVN cells and HCT116/IGF1-R transfectants were grown to confluence on a glass microscopy slide in the presence of 0.2% gelatin to eliminate floating cells. A “wound” was then created by scratching the slide with a razor blade, clearing a portion of the slide of adherent cells. Photo documentation was taken at days 1 and 4 after the wounding. At day 4, the migration of cells from the cut edge of the monolayer into the clear portion of the slide was assessed. The number of migrating cells was counted, using a Leitz Laborlux D microscope and a ×25 ocular, from the most cellular area at distances of 5, 10, and 15 mm from the cut edge of the monolayer.

Apoptotic Assay. To induce apoptosis HCT 116, HCT116/CVN, and HCT116/IGF1-R cells, grown in 6-well plates to 80% confluence, were placed in 10% and low serum (0.1%) medium, with or without the addition of 100 ng/ml IGF1 for 24 h. Apoptosis was determined by TUNEL using the in situ cell death detection kit (Boehringer Mannheim, Indianapolis, IN). Floating and attached cells were resuspended in PBS, and cytospin preparation were obtained. Cells were fixed with freshly prepared paraformaldehyde (4% in PBS, pH 7.4). Slides were rinsed with PBS, incubated in permeabilization solution, and cross-reacted with TUNEL reaction mixture for 60 min at 37°C in a humidified chamber. Slides were then rinsed again and incubated first with converter-alkaline phosphatase solution for 30 min at 37°C in a humidified chamber and later with alkaline phosphatase substrate solution for 5 to 10 min (Vector Laboratories, Burlingame, CA). After an additional rinse, the slides were mounted with coverslips and analyzed under a light microscope. The apoptotic rate was expressed as the number of apoptotic cells/2 microscopic fields using a Leitz Laborlux D microscope and a ×25 ocular. This experiment was performed in triplicate.

Tumorigenicity in Nude Mice. To assess the impact of IGF1-R overexpression on tumorigenicity, BALB/c-nu/nu mice (male, 7 weeks old) were injected s.c. with 7 × 106 HCT116 parental cells (six mice) or with the same number of HCT116/IGF1-R clonal cells (nine mice). Twenty-four hours before the injection, the cells were incubated in serum-free medium at 37°C. After trypsinization, cells were suspended in 0.2 ml of PBS and injected s.c. above
the hind leg of the nude mice at day 0. Tumor size was measured with a dial caliper, and the mice were euthanized at day 19. At necropsy, sections of the tumors and of the metastases were submitted for histological examination and TUNEL assay.

**Statistical Analysis.** The growth, soft agar, apoptotic, and migration experiment results obtained with the HCT116/IGF1-R clones were statistically evaluated and compared with those obtained with the respective HCT116 controls by the ANOVA test. The differences in tumor size were statistical analyzed by simple t test and by repeated measures ANOVA. Differences were considered significant if P < 0.05.

**RESULTS**

**HCT116/IGF1-R Transfectants Express Higher Levels of IGF1-R than HCT116 Parental Cells.** Our data show that HCT116/IGF1-R clonal cells express levels of IGF1-R that are up to 8-fold higher as compared with the parental HCT116 cells, by flow cytometry (56% versus 6.7%, respectively; Fig. 1A). Full-length IGF1-R cDNA expression constructs were also confirmed by PCR using primers spanning two exons (data not shown). In addition, the level of IGF1-R phosphorylation in HCT116/IGF1R clonal cells was ~5–10-fold higher than HCT116/CVN empty vector control cells (Fig. 1B).

**HCT116/IGF1-R Clonal Cells Have a Higher Proliferation Rate than HCT116 Parental Cells.** To study the relationship between the overexpression of a functional IGF1-R and cell proliferation, HCT116, HCT116/CVN, and HCT116/IGF1-R cells were subjected to [3H]thymidine incorporation assay. Although HCT116 parental cells and empty vector control cells (HCT116/CVN) did not respond to the addition of exogenous IGF1, the DNA synthesis in HCT116/IGF1-R clones to the added IGF1 was ~0.3-fold greater (Fig. 2A). Growth curve analysis revealed a ~2-fold increase in cell proliferation in the clonal cells (HCT116/IGF1-R) as compared with the parental cells (HCT116 and HCT116/CVN; Fig. 2B). This low proliferative response to the added IGF1 probably reflects the presence in these cells of an autocrine IGF1/IGF1-R stimulation. However, it is evident that HCT116/IGF1-R clonal cells grew at a higher rate than the wild-type cells or the empty vector control (HCT116/CVN; P < 0.005; Fig. 2A).

**HCT116/IGF1-R Transfectants Have Higher Transforming Activity than HCT116 Parental Cells.** To assess the hypothesis that HCT116/IGF1-R transfectants have a higher degree of anchorage-independent growth as compared with parental cells and to HCT116/CVN empty vector control, these cells were subjected to soft agar suspension assays. The results indicate that in the presence of 10% FBS, the clonal cells exhibited between 2- and 3-fold increase in colony formation in soft agar as compared with the parental (HCT116) and HCT116/CVN cells (Fig. 3A). We also noticed that in general, at any given time, the HCT116/IGF1-R colonies were on average 0.5 to 1.5 mm larger than the HCT116 and CVN/HCT116 colonies (data not shown). Remarkably, the treatment of HCT116/IGF1-R transfectants with αlR-3, 1 μg/60-mm plate for 2 h) before plating, and followed by subsequent supplementation with 20 ng/well of the same antibody, every 48 h for 14 days, resulted in significant inhibition in the formation of colonies in soft agar [HCT116/IGF1-R without αlR-3 = 123 colonies (±4); HCT116/IGF1-R with αlR-3 = 70 colonies (±9)] (Fig. 3B). These results indicate that IGF1-R is required for anchorage-independent growth of the human colorectal cancer cells under investigation.

**HCT116/IGF1-R Clonal Cells Display Increased Cell Motility Over the Wild-type HCT116 Cells.** Using an in vitro monolayer wounding assay, we observed that, when grown to confluence on glass microscope slides coated with 0.2% gelatin, a number of HCT116/IGF1-R clonal cells were able to migrate a distance of up to 15 mm from the cut edge of the monolayer (between 10 and 51 cells). By contrast, only one HCT 116 cell and five HCT116/CVN cells were able to migrate up to 10 mm away from the cut edge (Fig. 4). This difference was statistically significant (P < 0.0008 and <0.05 among the different clones). The differences in 5 mm and at 10 mm were also statistically significant (P < 0.0009 and <0.0004, respectively).

**HCT116/IGF1-R Cancer Cells Are Resistant to Serum Deprivation-induced Apoptosis.** HCT116/IGF1-R clonal cells are protected against serum deprivation-induced apoptosis, when the IGF1 ligand is added to the culture medium. Conversely, parental HCT116 cells and HCT116/CVN cells undergo significant apoptosis after incubation in 0.1% serum for 24 h, with or without the addition of IGF1 (between 5- and 10-fold more apoptotic cells as compared with

![Figure 5](https://example.com/fig5.png)
HCT116/IGF1-R Transfectants Express Higher Levels of Becl-\(x_1\) than the Parental HCT116 Cells. It has been suggested that IGF1-R may modulate the Becl-\(x_1\) expression (18). Here, we found that protection from apoptosis in HCT116/IGF1-R transfectants was associated with increased Becl-\(x_1\) expression, as compared with wild-type HCT116 cells that expressed only negligible levels of Becl-\(x_1\) (Fig. 7A). To determine the involvement of the Akt pathway in IGF1-R induced up-regulation of Becl-\(x_1\), IGF1-R stable clones were transfected with dominant negative Akt or pcDNA3 vector alone, as control (Fig. 7B). As shown in Fig. 7B, the expression of dominant negative Akt inhibited the IGF1-R-induced Becl-\(x_1\) expression.

Induction of becl-\(x_1\) Promoter by IGF1-R. HCT116/IGF1-R clonal cells and parental HCT116 cells were transiently transfected with a plasmid containing the luciferase gene driven by the becl-\(x_1\) promoter, together with dominant negative Akt, or vector alone, and stimulated with or without IGF1 (100 ng/ml). After 36 h of transfection, cells were lysed and subjected to luciferase reporter assay. Repeated experiments revealed that levels of luciferase activity in HCT116/IGF1-R clonal cells was ~3-fold higher than that of the parental cells transfected with the empty vector alone. Dominant negative Akt and attenuated IGF1-R-induced Becl-\(x_1\) promoter activity (Fig. 7C). However, no significant differences in luciferase activity were detected between the cell cultures in the presence and the absence of IGF1 (Fig. 7C). These data suggest that IGF1-R is able to activate Akt/Becl-\(x_1\) cascade and that autocrine secreted IGF1 is sufficient to activate the overexpressed IGF1-R and the Akt-dependent survival pathway.

HCT116/IGF1-R Transfectants Possess Increased Tumorigenicity as Compared with the IGF1-R–Parental Cells. When grown s.c. in nude mice, HCT116/IGF1-R cells were highly tumorigenic and acquired an aggressive behavior. IGF1-R transfectants formed tumors that were larger, when compared with those produced by HCT116 cells injected under the same conditions (Fig. 8). Evaluation by repeated measures ANOVA showed that there is a significant difference between the size of tumors from subject to subject (\(P = 0.001\)), but also between the size of the tumor at different time points (\(P < 0.0001\); Fig. 9 and Table 1).

Grossly and microscopically, these tumors infiltrated the adjacent skeletal muscle and spread within the abdominal cavity to adjacent and distant organs (Figs. 8, B and D and 10B). In contrast, mice injected with HCT116 cells revealed small tumors confined...
to the s.c. tissue (Fig. 8, A and C), and without evidence of local invasion or metastasis. A TUNEL assay revealed a larger number of apoptotic cells in HCT116 tumors (up to 40% of tumor) and only minimal apoptosis in HCT116/IGF1-R tumors (up to 5% of tumor; Fig. 10).

DISCUSSION

In the past decade, IGF1-R has emerged as a key regulator of mitogenesis and tumorigenicity, playing a crucial role in cell transformation, tumor invasion, and metastasis, and in cell survival (21–24, 41, 42).

IGF1-Rs have been detected in a decreasing gradient along the crypt-villous axis of normal intestinal mucosa (43). Although IGF1-R is overexpressed in a subset of human colon cancer (7–13), its ligands (IGF1 and/or IGF2) are usually expressed at higher levels in the neoplastic colonocytes than in the adjacent normal colonic mucosa (8). It has been observed that IGF1-R expression is 3–4-fold higher in primary colonic carcinomas than in adenomas and 2–3-fold higher in metastatic colon tumors than in primary tumors and that overall the level of expression of IGF1-R correlates with higher tumor stage (14). These findings are further supported by our data on the cell lines studied here, showing that colon cancer cells overexpressing the IGF1-R (HCT116/IGF1-R clonal cells) proliferate at higher rates than those not overexpressing this receptor (parental HCT116 cells). Furthermore, the degree of transformation, measured as number of col-
onies formed in soft agar suspension, is higher in the clonal cell lines, as compared with the parental cell line. The difference in soft agar clonogenic capability between HCT116 and HCT116/IGF1-R transfectants was accompanied by a constant larger size of the colonies formed by the latter. These findings support the view that an autocrine interaction between the IGF1-R and its ligands (IGF1 and/or IGF2) regulates both cell proliferation and anchorage-independent growth of colon cancer cells (9, 11, 13), and it has been shown that the transforming capability of IGF1-R may involve the activation of Src protein (44, 45). The essential role of IGF1-R in transformation is further supported by several investigations showing that the blockade of this receptor using either neutralizing antibodies or antisense strategies causes reversal of the tumorigenic phenotype in vitro and in vivo, for a wide variety of tumor types (26, 41, 42, 46–48), including colon cancer (49). This type of experimentation has revealed that IGF1-R is usually overexpressed in highly invasive and metastasizing tumors (50).

This association was also observed in the colon cancer cells studied here, where blocking of IGF1-R with a monoclonal antibody was able to partially revert the anchorage-independent growth of HCT116/IGF1-R cells in soft agar. In addition, the selected HCT116/IGF1-R transfectants were very motile and were highly tumorigenic in vivo, forming large invasive and metastasizing tumors, as compared with the parental cell line (HCT116). These findings are in agreement with those of others showing that antisense mRNA to IGF1-R and/or dominant negative mutants of IGF1-R inhibit the adhesion, invasion, and metastasis of cancer cells (49–54). It seems that IGF1-R down-regulates $M_{72,000}$ type IV collagenase (matrix metalloproteinase 2; Ref. 51), interacts with integrin receptors (52), and favors the adhesion of invading cells to laminin, a component of the cellular basement membrane (53, 54). These changes may be responsible for the migration of tumor cells across the extracellular matrix, facilitating invasion and metastasis.

Our experiments also show that the overexpression of a functional IGF1-R protects tumor cells from apoptosis. This finding is consistent with the proposed antiapoptotic role of the IGF1-R (27, 28). It has been shown that the antiapoptotic effect of the IGF1-R is independent of its mitogenic effect and that a mutation of tyrosine 1251 and/or replacement of histidine 1293 and lysine 1294 on the COOH terminus of the IGF1-R are able to abolish the receptor antiapoptotic function but not its transforming and mitogenic functions (29). The pathway used by the IGF1-R to protect cells from apoptosis have not been completely elucidated; however, there is evidence that the antiapoptotic function of IGF1-R is mediated via the activation of phosphatidylinositol 3-kinase (55), Akt/protein kinase B (56), and the phosphorylation and inactivation of BAD (57). Furthermore, it seems that, in colon cancer cells, the antiapoptotic effect of IGF1 depends on the TNF-α-induced activation of mitogen-activated protein kinase and nuclear factor-κB (30). IGF1-R-mediated protection from apoptosis may ultimately depend on the final threshold of signals resulting from the interaction of several complex pathways. For instance, Akt phosphorylates BAD which is unable to form heterodimers and thereby unable to neutralize the antiapoptotic effect of either Bcl-xL or Bcl-2 (58). Here, we show that activation of IGF1-R in the clonal cells is able to induce activation of Akt and up-regulation at the transcriptional level of $bcl-x_L$, with consequent increased expression of Bcl-xL protein. A similar finding has been reported in PC12 cells in which the addition of IGF1 results in significant increase of Bcl-x mRNA and Bcl-xL protein level (39), consequently preventing apoptosis. In our study, the up-regulation of Bcl-xL cor-

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Fig. 10. TUNEL assay on HCT116/IGF1-R and HCT116 tumors. HCT116 tumors (A and C) revealed extensive apoptosis, up to 40% of the tumor in some of the mice. Conversely, HCT116/IGF1-R clonal cells formed tumor infiltrating the adjacent skeletal muscle (B, arrows) had exhibiting only minimal apoptosis (up to 5% of the tumor; B and D).
related with the TUNEL experimental data, performed on sections of the tumors developed in nude mice, and showing that the HCT116/IGF1-R tumors had significantly less apoptosis than the HCT116 tumors.

In HCT116 cells, a phosphatidylinositol 3-kinase/Akt/Gsk-3 pathway has been shown to regulate apoptosis through modulation of TNF-related apoptosis-inducing ligand, a novel TNF family member (59). Nevertheless, the involvement of IGF1-R/Akt/Bcl-x pathway in survival of colon cancer cells has not been, to our knowledge, previously reported.

It is puzzling that although HCT116/IGF1-R clone 3 cells express more IGF1-R and have higher [3H]thymidine incorporation and cell survival of colon cancer cells has not been, to our knowledge, previously reported.

In conclusion, we report that the iatrogenic increase of IGF1-R in a parental colon cancer cell line (HCT116) originally expressing minimal levels of this receptor translated in a change of the malignant phenotype. The HCT116/IGF1-R transfectants, when compared with the parental cell line, exhibited a more aggressive phenotype, as demonstrated by their increased proliferative capacity, enhanced growth in soft agar, resistance to apoptosis, and increased tumorigenicity. In addition, we report that the antiapoptotic role of IGF1-R in these cells is associated with activation of Akt and up-regulation of Bcl-xL. Given the possible role of IGF1-R in determining an aggressive phenotype of colorectal cancer, the status of this receptor in resected human specimens may provide useful information to prescribe the most appropriate therapy for colon cancer patients.

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