Insulin Receptor Substrate-1 Regulates the Transformed Phenotype of BT-20 Human Mammary Cancer Cells

Ozlem Dalmizrak,1 An Wu,1 Jia Chen,1 Hongzhi Sun,1 Fransiscus E. Utama,1 Diana Zambelli,1,2 Thai H. Tran,1 Hallgeir Rui,1 and Renato Baserga1

1Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania and 
2Istituti Ortopedici Rizzoli, Bologna, Italy

Abstract

Although originating from a human breast cancer, BT-20 cells do not form colonies in soft agar. BT-20 cells do not express insulin receptor substrate-1 (IRS-1), which is known to promote both normal and abnormal growth and to inhibit differentiation. Stable expression of IRS-1 confers to BT-20 cells the ability to form colonies in soft agar. BT-20 cells form tumors in xenografts in mice, but the size of tumors is twice as large when the cells express IRS-1. The increased transformed phenotype is characterized by occupancy of the rDNA and cyclin D1 promoters by IRS-1 and the activation of the cyclin D1, c-myc, and rDNA promoters. In addition, the retinoblastoma protein, which is detectable in the rDNA promoter of quiescent BT-20/IRS-1 cells, is replaced by IRS-1 after insulin-like growth factor-1 stimulation. Our results indicate that in BT-20 human mammary cancer cells, expression of IRS-1 activates promoters involved in cell growth and cell proliferation, resulting in a more transformed phenotype. Targeting of IRS-1 could be effective in inhibiting the proliferation of mammary cancer cells. [Cancer Res 2007;67(5):2124–30]

Introduction

Insulin receptor substrate-1 (IRS-1) is a docking protein for both the insulin receptor and type I insulin-like growth factor receptor (IGF-IR). It binds to and activates phosphatidylinositol 3-kinase (PI3K) and, thus, plays an important role in signal transduction from the two receptors (1). IRS-1, especially when activated by the IGF-IR, sends a strong mitogenic, antiapoptotic, and antiodiferentiation signal (1, 2). IRS-1 expression is often increased in human cancer (3), and overexpression or ectopic expression of IRS-1 causes cell transformation, including the ability to form colonies in soft agar and tumors in mice (4, 5). When IRS-1 levels are decreased by experimental procedures (antisense or small interfering RNA), cancer cells lose their transformed phenotype (6–8). IRS-1 levels are low or even absent in the parental BT-20 breast cancer cells. IRS-1 increases colony formation in soft agar (20) and xenografts in mice. BT-20 cells originated from a human breast cancer, but they do not form colonies in soft agar or form only a few small ones (5, 18). BT-20 cells do not express IRS-1 (5) although they do express IRS-2 (see below). The absence of IRS-1 expression makes BT-20 cells an attractive model to test the mechanisms by which IRS-1 promotes cell transformation in mammary cancer cells. We have compared parental BT-20 cells to three BT-20–derived cell lines, all expressing IRS-1 by stable transfection with plasmids or retroviruses. BT-20/103 cells express modest amounts of IRS-1; BT-20/159 cells express high levels; and BT-20/NLS/IRS1 express an IRS-1 with a nuclear localization signal (19). We have used as criteria for transformation formation of colonies in soft agar (20) and xenografts in mice. Using these criteria, we show that, regardless of levels or localization, expression of IRS-1 in BT-20 cells increases colony formation in soft agar and tumorigenicity in mice. The increased transformation is accompanied by IRS-1 occupancy of the rDNA and cyclin D1 promoters; the dramatic activation of the rDNA, cyclin D1, and c-myc promoters; and the displacement by IRS-1 of the retinoblastoma tumor suppressor protein (pRb) from the rDNA promoter.

Materials and Methods

Cell lines and cell culture. BT-20 breast cancer cells were obtained from Eva Sarnac (Temple University, Philadelphia, PA) and cultured routinely in DMEM/Nutrient Mixture F-12 (Ham) 1:1 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% calf serum, glutamine, and antibiotics at 37°C in a 10% CO2 atmosphere. Media were supplemented with 2 μg/mL ampicillin for BT-20/NLS/IRS1 cells, 20 μg/mL hygromycin for BT-20/103 cells, and 0.5 μg/mL puromycin for BT-20/159 cells.

Stable transfection of BT-20 cells with the NLS-IRS-1 plasmid. Cells were transfected with pCMV/myc/nuc plasmid (Invitrogen, Carlsbad, CA) carrying a wild-type mouse IRS-1 cDNA at the Xho1/Not1 restriction site by electroporation. Nucleofector device and solutions were used (Amxaka, Gaithersburg, MD) using the program recommended by the manufacturer for this cell type.

Retroviral transduction of BT-20 cells with pGR103 and pGR159 plasmids. pGR103 and pGR159 plasmids were used for retroviral transduction of BT-20 cells. Plasmid pGR103 is a pMSCVhyg plasmid carrying wild-type mouse IRS-1 cDNA with its 3’ untranslated region (3’-UTR). Plasmid pGR159 is based on a self-inactivated form of the MSCV retroviral vector system and contains an internal cytomegalovirus promoter, a puromycin resistance gene, and wild-type mouse IRS-1 cDNA lacking the 3’-UTR (21). Transduction was carried out as previously described (21).

Cell growth and colony formation in soft agar. For monolayer growth, cells were seeded on 24-, 48-, and 72-h counting only the cells able to exclude trypan blue. Anchorage-independent growth was determined as previously described (5).

Xenografts. Animal experiments were done under approved Institutional Animal Care and Use Committee protocols. Intact female NCR athymic...
nude mice (N = 21; 7 mice per group; 8–9 weeks of age; obtained from Taconic, Hudson, NY) were inoculated s.c. with human BT-20, BT-20/159, or BT-20/IGF-IR/IRS1 cells (10⁶ suspended in 200 μL of 50% Matrigel (BD Biosciences, Bedford, MA) and 50% DMEM/F-12 (Invitrogen)) into each of two dorsolateral sites. Tumor growth was measured twice a week using a caliper and volume was calculated as 0.5 × (length × width × height), as described in detail by Tomayko and Reynolds (22).

**Western blots.** Western blots were carried out by standard procedures, as detailed in previous articles from this laboratory (4, 5, 13, 23).

**Chromatin immunoprecipitation assays.** Chromatin immunoprecipitation assays were carried out according to the manufacturer's instructions (Upstate, Lake Placid, NY) and the methods described by Chen et al. (19). The primers we used were as follows: rDNA promoter forward, 5'-TGTCTTGGTTGATGGAGG-3'; reverse, 5'-TCCGAGAAGGACGTCTGC-3'. Cyclin D1 forward, 5'-CGGACTAGGCTGTTGGTGTG-3'; reverse, 5'-CTCAGCATCGGTCGCGAGCAG-3'. For chromatin immunoprecipitation of pBh, we followed the procedure of Jackson and Pereira-Smith (24). Epicentre Fail Safe PCR System (Epicentre, Madison, WI) was used for PCR. The amplification products were analyzed in a 2% agarose gel and visualized by SYBR Gold (Molecular Probes, Eugene, OR) staining. Gels stained with SYBR Gold were scanned by using Typhoon Laser scanner (Typhoon 9400 Variable Mode Imager, Amersham Biosciences, Piscataway, NJ).

**Luciferase reporter assay.** To determine the activation of cyclin D1 (25) and c-myc (26) promoters by IGF-I, full-length pLuc3-U (obtained from Dr. Richard G. Pestell, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA) and pB-Luc (a kind gift of Dr. Bert Vogelstein, Howard Hughes Medical Institute and Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD) constructs were used, respectively. Cells (2 × 10⁵) were transiently transfected with 1 μg/well reporter plasmid by using nucleofector (Amaxa). To test transfection efficiency, cells were cotransfected with 50 ng/well of pRL-TK-Luc, a plasmid encoding Renilla luciferase (Promega, Madison, WI). The values were normalized to pRL-TK-Luc to generate relative luciferase unit.

**rDNA promoter activity.** The activity of the rDNA promoter was measured as described by Wu et al. (23).

**Glutathione S-transferase pulldown assays.** Glutathione S-transferase (GST) pull-down assays were carried out as previously described (19) for IRS-1, we synthesized GST constructs containing the following sequences: 1 to 300; 301 to 700; 701 to 1,000; and 1,000 to 1,234. A similar approach to affect the response to serum, which is determined by IGF-IR levels (27) and the presence of other growth factors. Figure 2 shows that colony formation in soft agar is dramatically different levels (27) and the presence of other growth factors. Figure 2 shows that colony formation in soft agar is dramatically different.

**Growth of BT-20–derived cells.** The cell lines of Fig. 1 were examined for their ability to grow in monolayer, in 1% or 10% serum, or in IGF-I. Serum definitely increases growth in all cell lines, with 10% serum being slightly better than 1% serum (Fig. 2A). The cells respond less to IGF-I, probably because the levels of IRS-1 (Fig. 1B). Fig. 1B also shows that IRS-2 is expressed in parental BT-20 cells (bottom).
few colonies in soft agar, the BT-20 cells expressing IRS-1 form a significant number of colonies, which are increased by IGF-I supplementation. The levels or subcellular localization of IRS-1 are unimportant; provided the cells express IRS-1, they form colonies in soft agar. The method we use for colony formation in soft agar is very stringent because we seed 2,000 cells per plate and only count colonies >125 μm in diameter after 3 weeks, as proposed in the original article (28). Parental BT-20 cells actually form very small colonies in soft agar, <125 μm in diameter, which are excluded from our stringent criteria. It is interesting that IGF-I, which is so weak in monolayer growth, can actually double the number of colonies in soft agar in cells expressing IRS-1.

**Tumor formation in nude mice.** We next determined the growth of BT-20–derived cells in nude mice. Parental BT-20 and BT-20 cells expressing IRS-1 (two clones) were tested for their ability to form tumors in nude mice (22). Tumor volumes were measured at various times after s.c. injection and the results are summarized in Fig. 3. BT-20 cells expressing IRS-1 form tumors that are twice as large as the tumors formed by parental BT-20 cells.

Because of the differences between monolayer growth and growth in soft agar or nude mice, we set out to investigate the mechanisms by which IRS-1 increases the transformed phenotype of BT-20 mammary cancer cells.

**Chromatin immunoprecipitation of the rDNA promoter.** Chen et al. (19) have shown that IGF-I can cause occupancy of the rDNA promoter by IRS-1. We have confirmed these results in BT-20–derived cells. Figure 4A shows a time course of IGF-I stimulation on the occupancy of the rDNA promoter. UBF is always present in the rDNA promoter (even in quiescent cells, there is always moderate rRNA synthesis), whereas IRS-1 occupancy is detectable only after 8 h of stimulation with IGF-I. This result is compatible with the original data by Tu et al. (13) that IRS-1 appears in the nuclei 8 h after stimulation, peaking at ~16 h. Figure 4 also shows the negative controls (we have omitted from the figure the control with GAPDH).

**Chromatin immunoprecipitation of the cyclin D1 promoter.** A similar experiment to the one described above for the rDNA promoter was carried out for the cyclin D1 promoter (Fig. 4B). Interestingly, UBF and IRS-1 are not detectable in the cyclin D1 promoter of BT-20–derived cells unless stimulated with IGF-I. Both proteins appear in the cyclin D1 promoter 8 h after stimulation with IGF-I. IRS-1 is absent from the cyclin D1 promoter of parental BT-20 cells, as expected.

**Effect of nuclear IRS-1 on the activity of the cyclin D1, c-myc, and rDNA promoters.** We next wanted to determine whether occupancy by IRS-1 of the rDNA and cyclin D1 promoters also had a functional effect on their activity. We added in this experiment the c-myc promoter. Parental BT-20 cells, BT-20 NLS-IRS1 cells, and BT-20/159 cells were transiently and separately transfected with two plasmids. The first has the cyclin D1 promoter

---

**Figure 2. Growth of BT-20 and BT-20–derived cells. A, growth in monolayer.** Cells were plated as described in Materials and Methods and stimulated as shown on the abscissa. Columns, unstimulated cells and cells on days 1, 2, and 3 after stimulation. B, colony formation in soft agar of BT-20 cell lines. The same cell lines were tested for colony formation in soft agar (see Materials and Methods). The colonies (>125 μm) were counted 3 wk after seeding. Light gray columns, colonies in 10% serum; dark gray columns, colonies in 10% serum supplemented with IGF-I (20 ng/mL).
driving luciferase (25); in the second plasmid (26), luciferase is driven by the c-myc promoter (see Materials and Methods). The presence of an nuclear IRS-1 markedly increases the activity of both cyclin D1 (Fig. 5A) and c-myc (Fig. 5A) promoters. The activity of the c-myc promoter peaks earlier than that of the cyclin D1 promoter, at 16 versus 24 h (Fig. 5).

For the activity of the rDNA promoter, we used the reporter gene described by Wu et al. (23), in which the rDNA promoter drives the expression of an SV40 T antigen. The plasmid was transiently transfected into parental BT-20 cells, BT-20/159 cells, and R+ cells that served as a positive control. IGF-I induces expression from the rDNA promoter in BT-20/159 cells but not in parental BT-20 cells (Fig. 5B). The levels of expression are comparable to those of R+ cells, a line of mouse embryonic fibroblasts in which IGF-I causes nuclear translocation of IRS-1 and increased RNA synthesis (13).

Activation of the cyclin D1 and c-myc promoters by serum. The experiments in Fig. 5A and B were done with cells stimulated by IGF-I. In the colony-forming assay, cells are in 10% serum, supplemented or not with IGF-I (Fig. 2). We therefore repeated the experiments by stimulating the cells with 10% serum. The results (Fig. 5C) were essentially the same. Serum activated both the cyclin D1 and c-myc promoters in BT-20/159 cells (expressing IRS-1) much more than in parental BT-20 cells.

IRS-1 and pRb in the rDNA promoter. It has been reported that pRb can be found in the nucleolus of quiescent or differentiating cells, where it binds UBF1 and causes a decrease in transcription from the rDNA promoter (29–31). It has also been reported that pRb binds to the pRb binding motif (LxCxE) of UBF1 (29), but subsequent reports have indicated that pRb binds to a different sequence of UBF1 (30). We first determined whether IRS-1 and pRb competed directly for binding to UBF1, and then we tested whether the expression of IRS-1 inhibited the occupancy of the rDNA promoter by pRb. We already knew indirectly that IRS-1 binds to UBF1 by its PHPTB domain (14). Figure 6A confirms directly that the PHPTB domain of IRS-1 is sufficient to immunoprecipitate UBF1 from cell lysates. We used the GST method (see Materials and Methods) to identify the UBF1 sequences required for IRS-1 binding. The NH2-terminal sequences were not necessary for binding of IRS-1 to UBF1 (not shown), which required instead the sequences located between residues 301 and 388 (Fig. 6B). Notice that in Fig. 6B, we also tried to identify the UBF1 sequences that bind PI3K, which phosphorylates UBF1 (32). PI3K binds the UBF1 sequences between residues 389 and 475. The GST experiments with pRb were not clear-cut as with IRS-1, but Fig. 6C shows that pRb binds preferentially to UBF1 sequences between 1 and 280, different therefore from the sequences binding IRS-1. We then proceeded to test the hypothesis that IRS-1 expression inhibits rDNA promoter occupancy by pRb.

The results (Fig. 6D) show that pRb is detectable in the rDNA promoter in parental BT-20 cells even after stimulation with IGF-I. In BT-20/159 and BT-20/NLS/IRS1 cells, pRb is detectable in the

Figure 3. Growth of BT-20–derived cells in xenografts. Parental BT-20 cells and BT-20 cells expressing IRS-1 (two clones; one of them also overexpressing the IGF-IR) were injected s.c. into nude mice and tumor volumes were determined as described in Materials and Methods. Columns, mean tumor volumes for each group; bars, SE. The experiment was terminated at day 32 but the tumors did not grow further between days 25 and 32.

Figure 4. Time course of rDNA and cyclin D1 promoter occupancy by IRS-1 in BT-20 cell lines. Chromatin immunoprecipitations were carried out as described in Materials and Methods. A, chromatin immunoprecipitation of the rDNA promoter in unstimulated cells (0) or cells stimulated with IGF-I for 8, 16, and 24 h. The sonicated chromatin was immunoprecipitated with antibodies to UBF and IRS-1. Top row, parental BT-20 cells; middle row, BT-20/NLS/IRS1 cells; bottom row, BT-20/159 cells. We have added the usual controls [IgGs and an antibody to growth factor receptor binding protein 2 (Grb-2), a cytoplasmic protein]. B, chromatin immunoprecipitation of the cyclin D1 promoter. The antibodies against UBF or IRS-1 and the control antibodies are indicated on top. Numbers above the lanes indicate the number of hours after IGF-I stimulation. Top row, parental BT-20 cells; middle row, BT-20/NLS/IRS1 cells; bottom row, BT-20/159 cells.
phenotype of BT-20 human mammary cancer cells. In addition, we have investigated some of the mechanisms by which IRS-1 can increase the transformed phenotype. We have used BT-20 cells because parental cells do not express IRS-1 (although they express IRS-2). The use of BT-20 cells is therefore very convenient for studying how IRS-1 expression affects the transformed phenotype.

Our results can be briefly summarized as follows: (a) expression of IRS-1 in BT-20 cells has very little effect on their ability to grow in monolayers, whether in serum or IGF-I, but it has a profound effect on their ability to form colonies in soft agar; (b) expression of IRS-1 increases the growth of BT-20 tumors in xenografts in mice; (c) IRS-1 in these cells translocate to the nuclei and can be found, by chromatin immunoprecipitation, in the rDNA and cyclin D1 promoters; (d) nuclear IRS-1 causes a marked activation of the rDNA, cyclin D1, and c-myc promoters, genes that are known to be involved in cellular proliferation; (e) the pRb protein, which binds and inhibits UBF1 (see above), is found in the rDNA promoter of quiescent cells, but it disappears from the promoter on stimulation by IGF-I and occupancy of the promoter by IRS-1; (f) although both pRb and IRS-1 bind to UBF1, they use different binding sites on the UBF protein. Whereas the effect of IRS-1 on the transformed phenotype is purely confirmatory, the mechanism involved (occupancy and activation of selected promoters) is novel.

There is an extensive literature on IRS-1 and its ability to promote growth (1, 2). IRS-1, activated by either the insulin

rDNA promoter at zero time, but disappears after stimulation with IGF-I, when IRS-1 is detectable in the rDNA promoter.

Discussion

The transformed phenotype of cell lines is characterized by a number of features of graded importance. According to the literature (20), the decreased requirement for growth factors (or serum) is the first step in transformation, followed by loss of contact inhibition (foci in monolayer cultures), acquisition of anchorage independence (colony formation in soft agar), and finally the ability to form tumors in experimental animals. We have used colony formation in soft agar and xenografts in mice to test whether the expression of IRS-1 could modulate the transformed

Figure 5. Activation of the cyclin D1, c-myc, and rDNA promoters in BT-20 cell lines. The indicated cell lines were transiently transfected (see Materials and Methods) with reporter plasmids driving luciferase. A, top row, the plasmid was pA3-Luc, where the cyclin D1 promoter drives luciferase; bottom row, the luciferase-driving promoter was the c-myc promoter pBV. For each cell line, the columns are zero time, 8, 16, and 24 h after IGF-I. Luciferase activity was measured at the indicated hour after transfection and stimulation with IGF-I (50 ng/mL). B, activation of the rDNA promoter. Parental BT-20 cells (lanes 1–4), BT-20/159 cells (lanes 5–8), and R+ cells (lane 9) were transfected in transient with the appropriate plasmid (see text) and were left unstimulated or were stimulated with IGF-I for the times (in hours) indicated above the lanes. Western blot with an antibody to SV40 T antigen, driven by the rDNA promoter. C, activation of the cyclin D1 and c-myc promoters by serum. Parental BT-20 cells and BT-20/159 cells were made quiescent in serum-free medium and were then stimulated with 10% serum for the indicated times. The activity of the cyclin D1 (top) and c-myc (bottom) promoters was then determined by the luciferase assay as in (A).

Figure 6. Interactions of pRb and IRS-1 with UBF and occupancy of the rDNA promoter. A, immunoprecipitation of UBF1 by an IRS-1 expressing only the pleckstrin and phosphotyrosine binding domains (16). B, GST analysis of UBF binding to IRS-1 and PI3K. The procedure is described in Materials and Methods. Pulldown assay with 500 μg of lysates and blotting with antibodies to IRS-1 and PI3K. Lane 1, empty GST; lane 2, UBF1 residues 301 to 388; lane 3, residues 389 to 475; lane 4, residues 476 to 550; lane 5, R+ lysates; lane 6, 32-d cell lysates. C, GST analysis of UBF binding to pRb. Pulldown assay with 500 μg of lysates and blotting with an antibody to pRb, diluted 1:1,000. Lane 1, empty GST; lane 2, UBF1 residues 1 to 280; lane 3, residues 281 to 560; lane 4, residues 561 to 670; lane 5, residues 300 to 600; lane 6, BT-20 whole-cell lysate. D, chromatin immunoprecipitation of the rDNA promoter in BT-20 and BT-20-derived cells. Immunoprecipitation with an antibody to pRb and PCR for the rDNA promoter. The cell lines and the times (in hours) after stimulation with IGF-I are indicated above the lanes.
receptor or the IGF-IR, activates the PI3K pathway (33), which is known to be one of the main signaling pathways for cellular proliferation (34, 35). A constitutively active catalytic subunit of PI3K transforms cells in culture, and there are reports of mutated, oncogenic PI3K in human tumors (36). IRS-1 per se is also known to be a strong mitogen and to inhibit differentiation (3–5). Down-regulation of IRS-1 (by either antisense or small interfering RNA) abrogates the transformed phenotype (6–8). IRS-1 plays a major role in the regulation of cell size (2, 4, 37), and deletion of IRS-1 genes in mice (38) or Drosophila (39) causes a 50% reduction in body size. The reduction in body size is due both to decreased cell size and decreased cell number (40). Ectopic expression of IRS-1 doubles the size of myeloid cells (4, 37). This effect on cell size is explained by the finding that IRS-1 activates UBF1 (13, 14, 23), a protein that regulates the activity of RNA polymerase I and, therefore, cell size (15).

Our communication, however, uncovers another aspect of IRS-1, specifically its ability to promote growth by translocating to the nuclei and acting directly on the rDNA, c-myc, and cyclin D1 promoters. Nuclear translocation of IRS-1 has been reported in cells in culture (12–14) and in vivo in medulloblastomas (11), human breast cancer (16, 17), and rat liver (41). We show here that IRS-1 can actually be found in the rDNA and cyclin D1 promoters. Its presence there, and presumably in the c-myc promoter, causes a marked activation of the three promoters, whether by IGF-1 or by serum.

There are many reports of growth factors, growth factor receptors, and signal transduction molecules accumulating in the nuclei. Some of them, such as the epidermal growth factor receptor, fibroblast growth factor (FGF)-2, and FGF receptor-1, may act as transcriptional cofactors [refs. 42–45; reviewed by Massie and Mills (46)]. In the experiments of Sheng et al. (47), FGF-2 was shown to activate the rDNA promoter in a way similar to our demonstration that IRS-1 activates it. Liangli et al. (48) found a nuclear fragment of ErkB-4 with an active tyrosine kinase activity. The finding that IRS-1 binds to UBF1 and stimulates rRNA synthesis (13, 14) is in itself a strong suggestion of IRS-1 acting as a transcriptional cofactor. In fact, it is not surprising that IRS-1 moves to the promoters of genes that it activates. A recent report by Pokholok et al. (49) has indicated that, in yeast, activated signal transduction kinases can be found in the promoters of genes they regulate. This pattern may be more common than it is at present realized.

The crucial observation in these experiments is that ectopic expression of IRS-1 has little effect on the growth of BT-20 cells in monolayer cultures. It has, however, a dramatic effect on the growth of BT-20 cells in soft agar and increases tumor growth in xenografts. The increased transformation of parental BT-20 cells by IRS-1 is accompanied by occupancy of the rDNA and cyclin D1 promoters by IRS-1 and by the activation of the rDNA, c-myc, and cyclin D1 promoters. Because these genes are all heavily involved in cell proliferation, the effect of nuclear IRS-1 on their expression provides one explanation for the transforming activity of IRS-1 in these mammary cancer cells.

The results in the present communication confirm the important role of IRS-1 in growth and transformation, especially of a nuclear IRS-1. The presence of IRS-1 in the rDNA and cyclin D1 promoters (and also in the c-myc promoter) suggests the possibility that IRS-1 in the nuclei may act as a cotranscriptional cofactor for both RNA polymerase I– and RNA polymerase II–directed transcripts. The results of Fig. 6 suggest an explanation. The literature has shown that pRb can bind UBF1 and inhibit it (see above) whereas IRS-1 binds also to UBF1 but activates it (13, 14). We show here that, as expected, pRb can be found in the rDNA promoter of BT-20 cells. In BT-20 cells expressing IRS-1, pRb is present in the rDNA promoter only at zero time, and disappears after IGF-I stimulation (which causes occupancy by IRS-1; see Fig. 4).

The significance of these findings is that IRS-1 seems to be a good candidate for targeting if one wishes to decrease cell proliferation and increase differentiation. Whereas IRS-1 acts usually through PI3K, the advantage of targeting IRS-1 is that abrogation of IRS-1 expression inhibits proliferation and favors differentiation of normal cells but does not necessarily kill them, whereas abrogation of the catalytic subunit of PI3K is a lethal phenotype (50).

In conclusion, we have shown that in BT-20 mammary cancer cells, IRS-1 expression markedly increases the transformed phenotype, and that this effect is accompanied by occupancy and activation by IRS-1 of the rDNA, cyclin D1, and c-myc promoters and the displacement of pRb from the rDNA promoter.

Acknowledgments

References

Insulin Receptor Substrate-1 Regulates the Transformed Phenotype of BT-20 Human Mammary Cancer Cells

Ozlem Dalmizrak, An Wu, Jia Chen, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/5/2124

Cited articles
This article cites 45 articles, 22 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/5/2124.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/67/5/2124.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.