Microarray Analysis and Identification of Novel Molecules Involved in Insulin-like Growth Factor-1 Receptor Signaling and Gene Expression

JOELLE DUPONT,* SANDRA E. DUNN,† J. CARL BARRETT,‡ AND DEREK LEROITH¶

Physiologie de la Reproduction et des Comportements, Unite Mixte de Recherches 6073, Institut National de la Recherche Agronomique-Centre National de la Recherche Scientifique, Université F. Rabelais, Tours 37380 Nouzilly, France; †British Columbia Research Institute for Children's and Women's Health, Vancouver, British Columbia, Canada; ‡Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892-2440; ¶Clinical Endocrinology Branch, National Institutes of Health, Bethesda, Maryland 20892-1758

ABSTRACT

The insulin receptor (IR) and the insulin-like growth factor-1 receptor (IGF-1R) are members of the same subfamily of receptor tyrosine kinases. The two receptors phosphorylate many of the same substrates and activate the same signaling modules, including the mitogen-activated protein kinase (MAPK) and phosphatidyl inositol 3′ kinase (PI3K) signaling pathways. Although the IR and IGF-1R share some redundant functions in metabolism, cell growth, differentiation, and apoptosis, they also exhibit distinct physiological roles. Some of these may be due to differences in tissue distribution, receptor structure, formation of hybrid receptors, or mechanisms of ligand binding. However, the divergent effects of insulin and IGF-1 also may be explained by specificity in the intracellular signals generated by insulin and IGF-1. In particular, the IR and IGF-1R are capable of triggering their own biological responses by using specific or preferential substrates, molecular adapters, or signaling pathways. In a recent study, we used cDNA microarray analysis to identify genes differentially regulated by insulin and IGF-1. Mouse NIH-3T3 fibroblasts expressing either the wild-type human IGF-1R or IR were stimulated with either IGF-1 or insulin, respectively. We identified 39 genes differentially regulated by insulin and IGF-1. Most of these genes had not been reported previously to be responsive to insulin or IGF-1. The genes induced by IGF-1 generally were involved in mitogenesis or differentiation, while the genes found to be induced by insulin did not conform to any particular category. In a separate study, immortalized breast epithelial cells were stimulated with IGF-1 and a cDNA microarray analysis was used to generate a profile of IGF-1-regulated genes. A number of genes known to be involved in angiogenesis were found to be regulated by IGF-1. These results strongly suggest that this technology may be extremely useful in identifying groups of genes that are specifically regulated by different ligands and their activated receptors.

I. Introduction

Insulin and insulin-like growth factor-1 (IGF-1) are peptide hormones that are homologous in primary structure but differ in their physiological effects.

Insulin, produced by the beta (β) cells of the pancreas, stimulates the uptake of glucose and amino acids, inhibits gluconeogenesis, and promotes lipogenesis. IGF-1 is involved primarily in cell growth, survival, apoptosis, and differentiation. Insulin and IGF-1 mediate their biological effects by binding to their respective receptors, the insulin receptor (IR) and the IGF-1 receptor (IGF-1R). Although IR and IGF-1R are structurally and functionally similar, targeted gene knockouts in mice showed that they have both overlapping and distinct physiological roles (Nakae *et al.*, 2001). One of the major areas of interest in this field is to understand how the specificity of IR and IGF-1R signaling is defined. In this review, we briefly compare and contrast the IR and IGF-1R signaling pathways and discuss various mechanisms that could explain the divergent physiological functions mediated by the two receptors. We then describe recent experiments using cDNA microarray analysis that have identified specific differences at the level of gene expression.

II. Structure of the Insulin and IGF-1 Receptors

The IR and IGF-1R are both comprised of two extracellular alpha (α) subunits containing ligand-binding sites and two transmembrane β subunits transmitting the ligand-induced signal (Yarden and Ullrich, 1988). More specifically, IGF-1R and IR β subunits consist of three domains: 1) a juxtamembrane domain, with motifs required for recruiting the major signaling adapter proteins; 2) a tyrosine kinase domain, essential for catalytic activity of the receptor; and 3) the carboxyl-terminal domain, which has several important residues for IGF-1R and IR signaling (Figure 1). As a consequence of this high level of homology, hybrid receptors, comprised of an insulin $\alpha\beta$-hemireceptor and an IGF-1 $\alpha\beta$-hemireceptor, can form in tissues and cultured cells expressing both the IR and the IGF-1R (Federici *et al.*, 1997). Such hybrid receptors may play a role in the divergent actions of insulin and IGF-1.

A. EXTRACELLULAR (LIGAND-BINDING) DOMAIN

Despite the structural similarities between IGF-1 and insulin, the IR and IGF-1R have 100- to 1000-fold higher binding affinity for their cognate ligands. The α subunits have been shown to confer ligand-binding specificity (Schumacher *et al.*, 1991). Some studies using chimeric receptors have shown that the high affinity of the IR for insulin is determined by regions adjacent to the cysteine-rich domain (Gustafson and Rutter, 1990; Schumacher *et al.*, 1991). On the other hand, the high-affinity IGF-1 binding by the IGF-1R is determined by its cysteine-rich domain within the α subunit.

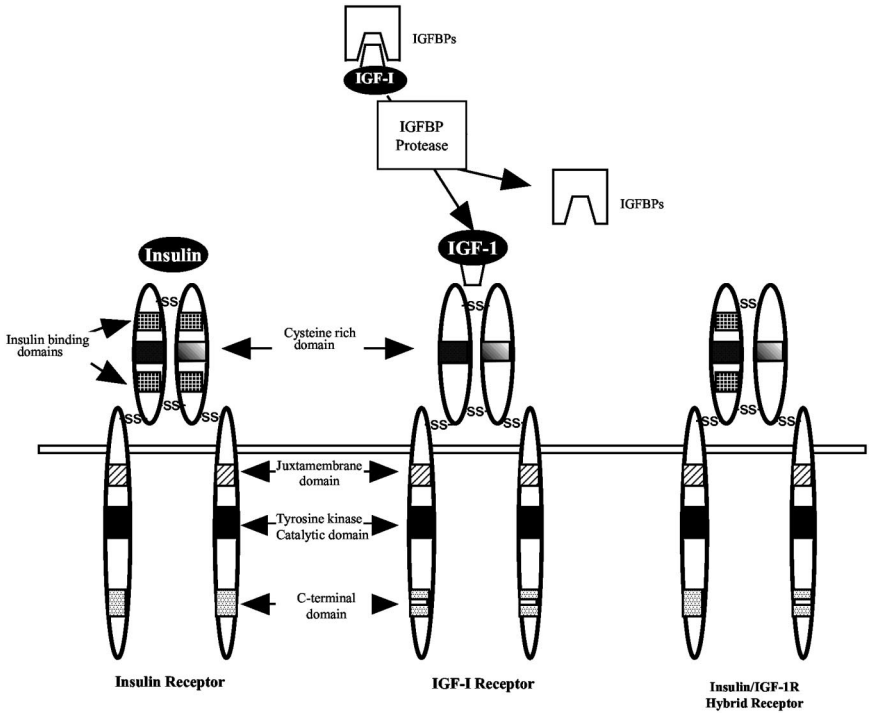

FIG. 1. The insulin-like growth factor (IGF) family of ligands, binding proteins (IGFBPs), and receptors (IGF-1R). The IGFs are bound by binding proteins in the circulation and in the extracellular matrix of the target cells. One mechanism for their release is specific protease effects on the IGFBPs, which release the IGFs to interact with their specific cell-surface receptors. IGFs may interact with IGF-1, insulin, and hybrid receptors to affect cell signaling within the target cell.

B. CYTOPLASMIC DOMAIN

Structural differences in the cytoplasmic domain of the β subunits of the IR and IGF-1R may contribute to the divergence of these two signaling pathways. The highest degree of homology between the two receptors is found within the tyrosine kinase domain (about 84%), whereas the region of greatest divergence between the IR and IGF-1R is found within the juxtamembrane domain (about 61%) and the carboxyl-terminal domain (about 56%) (Ullrich *et al.*, 1986; Ullrich and Schlessinger, 1990). Chimeric receptors consisting of the ligand-binding domain of IR and the cytoplasmic domain of IGF-1R functioned more like the IGF-1R than the IR (Lammers *et al.*, 1989). Similarly, chimeric IGF-1R containing the carboxyl-terminal β subunit domain of the IR more closely resembled the IR than the IGF-1R (Tartare *et al.*, 1994). To eliminate interactions of ligands with endogenous receptors, other chimeras were generated in which

the extracellular portion of the neurotrophin receptor was fused to the intracellular portions of IR or IGF-1R (Siddle *et al.*, 2001). These chimeric molecules were stably expressed in 3T3-L1 fibroblasts (Kalloo-Hosein *et al.*, 1997) or 3T3-L1 adipocytes (Urso *et al.*, 1999,2001) at levels comparable to those of endogenous IR or IGF-1R and activated by nerve growth factor (NGF). The TrkC-IR chimeric receptor was more effective in stimulating physiologically relevant metabolic responses, whereas the TrkC-IGF-1R was more effective in promoting mitogenesis (Urso *et al.*, 1999). Thus, the intracellular domains of the IR and IGF-1R are likely to mediate at least part of the observed receptor specificity.

III. Signal Transduction via IR and IGF-1R

A. COMMON SIGNALING PATHWAYS

Many of the intracellular signaling events mediated by activation of the IR and IGF-1R are remarkably similar (White, 1994; Cheatham and Kahn, 1995; LeRoith *et al.*, 1995) (Figure 2). Some of the shared substrates that become phosphorylated by the IGF-1R and IR include members of the insulin receptor substrate (IRS) family of proteins (IRS-1, -2, -3, and -4) (Sun *et al.*, 1991; Lavan and Lienhard, 1993; Patti *et al.*, 1995; Fantin *et al.*, 1998), Gab-1 (Winnay *et al.*, 2000), and Shc (Pelicci *et al.*, 1992). Upon stimulation by insulin or IGF-1, tyrosine-phosphorylated IRS and Shc proteins form signaling complexes between phosphotyrosine-containing binding motifs (YXXM) and Src homology 2 (SH2) domains found in molecules such as growth factor receptor binding-2 protein (Grb2) (Lowenstein *et al.*, 1992; Skolnik *et al.*, 1993) and the p85 regulatory subunit of the phosphatidyl inositol 3′ kinase (PI3K) (Backer *et al.*, 1992). The phosphotyrosine residues on IRS-1 also form docking sites for other signaling molecules, including Syp (SHPTP2) (Xiao *et al.*, 1994), Fyn (Sun *et al.*, 1996), Nck (Lee *et al.*, 1993), and Crk (Beitner-Johnson *et al.*, 1996).

By binding to Grb2, IRS proteins couple the IR and IGF-1R to the Ras/mitogen-activated protein kinase (MAPK) pathway. This pathway regulates cell growth, differentiation, and proliferation in response to insulin and IGF-1 (Blenis, 1993; Crews and Erikson, 1993). Various protein tyrosine phosphatases can regulate the activities of the IR and IGF-1R signaling systems.

B. SPECIFICITY

1. Proximal Substrates

To understand the mechanisms involved in the distinct physiological functions of insulin and IGF-1, some investigators searched for specific substrates for

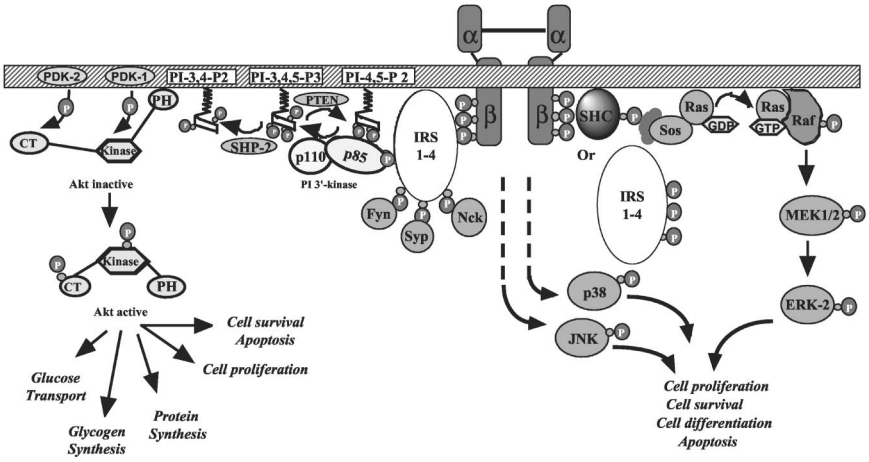

FIG. 2. Multiple signaling pathways for the IGF-1 receptors. These include the Ras/Raf/mitogen-activated protein (MAP) kinase pathways that lead to cell proliferation. For example, the phosphatidyl inositol 3′ kinase (PI3K) pathway also has multiple effects and other pathways such as the p38 MAP kinase and Jun kinase (JNK) pathways also affect these biological outcomes. Abbreviations: CT, carboxy-terminal; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; IRS, insulin receptor substrate; MEK, mitogen extracellular kinase; PDK, phosphoinositide-dependent kinase; PH, pleckstrin homology domain; PI, phosphatidylinositol; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SHC, Src homology collagen; SHP, Src homology phosphatase.

IR or IGF-1R. Najjar and coworkers identified pp120, a plasma membrane glycoprotein, which is a substrate for the IR but not for the IGF-1R (Najjar *et al.*, 1997; Soni *et al.*, 2000). Phosphorylation of pp120 is required for its function in insulin endocytosis (Formisano *et al.*, 1995), bile acid transport (Sippel *et al.*, 1994), tumor suppression (Kleinerman *et al.*, 1995), and its inhibitory effect on the mitogenic actions of insulin (Soni *et al.*, 2000). Interestingly, when the carboxyl-terminus of the IGF-1R is replaced by an equivalent region of the IR, the chimeric IGF-1R then can bind to and phosphorylate pp120, decreasing its effect on cell growth (Soni *et al.*, 2000). Mutation of the tyr^{1316} in the IR, which is not conserved in the IGF-1R, abrogates the insulin-induced tyrosine phosphorylation of pp120 and its ability to suppress the mitogenic action of insulin (Soni *et al.*, 2000).

Some of the other substrates of the IR and IGF-1R are differentially phosphorylated in response to IGF-1 or insulin, indicating that they may mediate specific effects for both ligands. The molecular adapter Grb14 binds specifically to the regulatory kinase loop of the IR and inhibits catalytic activity (Kasus-Jacobi *et al.*, 1998). It recently was shown that Grb14 is three to 10 times less effective at inhibiting the catalytic activity of the IGF-1R than the IR (Bereziat

et al., 2002). Rother and coworkers showed that the specificity of signaling may be explained by the preferential use of different substrates by the IR and IGF-1R (Rother *et al*., 1998). In particular, the IR was coupled preferentially to IRS-2, whereas the IGF-1R was coupled preferentially to IRS-1. This conclusion was confirmed by ablation of the IRS-1 and IRS-2 genes in mice (Araki *et al.*, 1994; Tamemoto *et al.*, 1994; Withers *et al.*, 1998).

In Chinese hamster ovary (CHO) cells stably expressing either the human IR or IGF-1R, it was shown that there are differences in the complement of SH2-containing proteins recruited to IRS-1 by the two receptors (Amoui *et al.*, 2001). In particular, the IGF-1R appears to couple IRS-1 preferentially to Grb2, whereas the IR appears to couple IRS-1 preferentially to the p85 subunit of PI3K (Amoui *et al.*, 2001). In other recent studies, Olefsky and coworkers showed that both the IGF-1R and IR can function as G protein-coupled receptors and engage different G-protein partners. The IGF-1R utilizes Gαi, whereas the IR does not (Dalle *et al.*, 2001). In contrast, the IR signals through Gαq/11, whereas the IGF-1R does not (Imamura *et al.*, 1999; Dalle *et al.*, 2001). Using the two-hybrid system, Grb10 was found to associate preferentially with the IR in mouse fibroblasts expressing either the IR or IGF-1R (Laviola *et al.*, 1997). Using the same technique, the protein 14-3-3β was found to bind to the IGF-1R but not to the IR (Furlanetto *et al.*, 1997).

Recently, Ligensa and coworkers identified a new PDZ (postsynaptic density protein-95, disc large, zonula occlusions-1) domain-containing protein (IGF-1 receptor interacting protein-1, IIP-1) that interacts with the C-terminal tail of the IGF-1R but not the IR (Ligensa *et al.*, 2001). Furthermore, the most distal three amino acids in the C-terminal tail of the IGF-1R appear to be crucial for the interaction of IIP-1 with IGF-1R. Indeed, a mutated IR tail carrying the terminal three amino acids of the IGF-1R is able to bind to IIP-1, whereas mutating any of the terminal three amino acids in the IGF-1R tail to the corresponding three amino acids in the IR abolishes the interaction with IIP-1 (Ligensa *et al.*, 2001). Overexpression of IIP-1 in MCF-7 cells does not affect either IGF-1-dependent proliferation or IGF-1-mediated protection from apoptosis but significantly reduces cell motility (Ligensa *et al.*, 2001). Hermanto and coworkers also identified a novel IGF-1R-interacting molecule called RACK1 (Hermanto *et al.*, 2002). RACK1 associates specifically with the IGF-1R but not with the IR, both in yeast and in HEK293T and NIH-3T3 cells overexpressing either the IGF-1R or the IR (Hermanto *et al.*, 2002). RACK-1 is involved in IGF-1R-mediated regulation of cell growth and transformation (Hermanto *et al.*, 2002). However, the interaction of RACK1 with the IGF-1R but not the IR seems to be specific to certain cell types. Indeed, Kiely and coworkers showed that endogenous RACK-1 could interact with both the endogenous IGF-1R and IR in Chinese ovary siemens (COS) cells (Kiely *et al.*, 2002). Nevertheless, these different

receptor-specific adaptor proteins, particularly IIP-1 and RACK1, might contribute to the biological specificity of the two hormones.

2. Signaling Pathways

Some evidence suggests that the IR and IGF-1R may phosphorylate the same substrates but use different signaling pathways to mediate the same or different biological effects. For example, insulin induces the expression of vascular endothelial growth factor (VEGF) via the PI3K/Akt pathways in NIH-3T3 cells overexpressing the human IR, whereas IGF-1 induces VEGF expression via the mitogen extracellular kinase (MEK)/MAPK pathway in NIH-3T3 cells overexpressing human IGF-1R (Miele *et al.*, 2000). In rat hepatic stellate cells, insulin and IGF-1 both stimulate cellular proliferation. However, both PI3K and extracellular signal-regulated kinase (ERK) are involved in IGF-1-induced mitogenesis, whereas insulin stimulated mitogenesis through a PI3K-dependent and ERK-independent pathway (Svegliati-Baroni *et al.*, 1999). Interestingly, glycogen synthesis was more effectively stimulated by the IR than by the IGF-1R, although both receptors mediated similar activation of the Akt/protein kinase B (PKB) protein kinase in hepatocytes and in 3T3-L1 fibroblasts (Park *et al.*, 1999). The insulin-specific stimulation of glycogen synthesis appears to involve a rapamycin-sensitive pathway in hepatocytes (Park *et al.*, 1999).

To explain the specificities in the function of the IR and IGF-1R, some investigators suggested that downstream kinases may mediate the specific effects of the IR vs. the IGF-1R. For example, Nakae *et al.* reported that the transcription factor forkhead homologue to rhabdomyosarcoma (FKHR) is differentially regulated by insulin and IGF-1 in hepatocytes. The phosphorylation of one threonine residue in particular (Thr-24) appears to be induced by insulin but not by IGF-1. As this residue can be phosphorylated by PKB *in vitro* and PKB is also activated by IGF-1 in these cells, the authors proposed that a PKB-like kinase specifically activated by insulin may mediate this effect (Nakae *et al.*, 2000). More recently, it has been shown that although both insulin and IGF-1 induce proliferation of murine skin keratinocytes, the action of insulin — but not IGF-1 — is mediated specifically via a protein kinase C delta (PKCδ) and involves activation of the sodium/potassium (Na^+/K^+) pump (Shen *et al.*, 2001). Thus, PKCδ is a multifunctional serine kinase that represents a divergence point in IR and IGF-1R signaling. In this same cell type, insulin and IGF-1 stimulate the translocation of different glucose transporters, although they both increase glucose uptake (Shen *et al.*, 2001). Thus, insulin and IGF-1 can mediate the same or different biological responses by utilizing different signaling pathways or different intracellular mediators.

IV. Induction of Specific Genes by the IR and IGF-1R

Some reports have shown that insulin and IGF-1 can act on the same genes but with different outcomes. For example, in murine skin keratinocytes, insulin stimulates the expression of differentiation markers, whereas IGF-1 inhibits them (Wertheimer *et al.*, 2000). Also, in the developing eye lens of the chicken, the level of delta-crystallin induced by IGF-1 is greater and occurs more quickly than that induced by insulin (Alemany *et al.*, 1989). It has been shown that low concentrations of IGF-1 (10 nM) increase the expression of uncoupling protein 3 (UCP-3) by 2-fold, whereas much higher concentrations of insulin (860 nM) are necessary to obtain the same effect in human neuroblastoma SH-SYSY cells (Gustafsson *et al.*, 2001).

cDNA microarray analysis recently has been established as a powerful tool to study the effects of hormones on cellular metabolism and gene regulation on a genomic scale. Until now, this technology was used to define the effects of IGF-1 on gene expression in different cell lines (Liu *et al.*, 2001; Oh *et al.*, 2002) but not to compare the different gene-expression profiles induced by insulin and IGF-1. We used cDNA microarray expression profiling to identify genes that are regulated differently by IGF-1 and insulin in mouse fibroblast NIH-3T3 cells (Dupont *et al.*, 2001b) as a first step towards understanding the molecular basis for the different functions of the IGF-1R and the IR.

A. DIFFERENTIAL REGULATION OF GENE-EXPRESSION PATTERNS BY INSULIN AND IGF-1 IN NIH-3T3 FIBROBLASTS

The biological and physiological comparison of the IR and IGF-1R is complicated by the fact that each ligand can cross-react with the other receptor and hybrid receptors can form when both receptors are expressed in the same cells. To circumvent these problems, we have compared the effect of insulin and IGF-1 in NIH-3T3 fibroblasts overexpressing either human IR (IR cells) (Levy-Toledano *et al.*, 1993) or human IGF-1R (NWTb3 cells) (Blakesley *et al.*, 1995,1996). NWTb3 and IR cells were incubated in the presence or absence of IGF-1 (50 nM) or insulin (50 nM) for 90 minutes, respectively. Of the 2221 genes on the mouse cDNA microarrays, we found that the expression levels of 30 were significantly induced by IGF-1 but not by insulin. In contrast, only nine genes and one expressed sequence tag (EST) were upregulated specifically by insulin but not by IGF-1 (Tables I and II). We confirmed the IGF-1- and insulin-induced regulation for 10 of these genes by Northern analysis (Figure 3). The genes that were identified as regulated by IGF-1 and insulin are involved in various cellular functions, including proliferation, differentiation, apoptosis, cellular processes, and metabolism (Tables I and II). Interestingly, most of these genes were not known previously to be regulated by either IGF-1 or insulin. Indeed, only three genes — the Jun oncogene (Chiou and Chang, 1992; Monnier *et al.*, 1994), α5

integrin (Palmade *et al.*, 1994), and the early growth response-1 transcription factor (EGR-1) (Jhun *et al.*, 1995) — had been reported to be induced by IGF-1. Furthermore, more than half of the genes upregulated by IGF-1 are associated with mitogenesis and differentiation, whereas none of the genes specifically upregulated by insulin are associated with these processes. IGF-1, but not insulin, induced the expression of two cytokine receptors (interleukin (IL) receptors 3 and 4) that have been reported to be involved in the regulation of cell growth (Keegan *et al.*, 1994). IGF-1 also induced the expression of glial cell line-derived neurotrophic factor (GNDF), which is known to be crucial for the development and the maintenance of various neurons (Airaksinen and Saarma, 2002). IGF-1 increased the expression of the Wee-1-like kinase, which is involved in cell-cycle progression (Helmbrecht *et al.*, 2000), and the EGR-1 transcription factor, which is known to enhance cell proliferation. These results suggest that IGF-1-induced cellular proliferation is a tightly regulated process.

Our study also suggested that insulin and IGF-1 are involved in the apoptosis process. IGF-1 treatment increased expression of the T-cell death-associated gene (TDAG)-51 and Daxx (Fas-binding) genes, whereas insulin increased expression of apoptotic protease-activating factor-1 (APAF-1) and seven in absentia homologue-1B (SIAH-1B) (Tables I and II). Importantly, IGF-1 is capable of increasing the expression of antiapoptotic genes such as Twist (Maestro *et al.*, 1999). Thus, the induction of IGF-1- or insulin-specific genes could explain the specificity of the biological effects of these two hormones.

B. TWIST EXPRESSION IS SPECIFICALLY INDUCED BY IGF-1

In a separate study, we studied Twist, one of the genes that was specifically induced by the IGF-1-responsive gene (Dupont *et al.*, 2001a). Twist belongs to the basic helix-loop-helix family of transcription factors, which play a central role in cell-type determination and differentiation in both vertebrates and invertebrates (Olson and Klein, 1994). IGF-1 treatment increased the abundance of Twist mRNA in NWTb3 cells, whereas insulin failed to increase Twist mRNA in IR cells. The IGF-1-induced increase in Twist expression requires activation of IGF-1R, since Twist mRNA expression was not induced in response to IGF-1 in parental NIH-3T3 cells, which express few IGF-1Rs, nor in the NKR (NIH-3T3 cells expressing an IGF-1 receptor with lysine-to-arginine substitution) cell line, which overexpresses the dominant-negative human IGF-1R (Kato *et al.*, 1993). We also showed that injection of IGF-1 via the inferior vena cava increased Twist mRNA expression in muscle. We used various pharmacological inhibitors and a MEK-1 dominant-negative construct to investigate which IGF-1R signaling pathway was involved in the induction of Twist gene expression. These experiments demonstrated that the MEK/MAPK pathway plays a critical role in IGF-1-induced Twist expression. Using an antisense strategy, we

TABLE I
Genes That Are Specifically Upregulated by Insulin-like Growth Factor-1 in NIH-3T3 Fibroblasts

	Symbol	Clone number	IGF-1	Insulin
Mitogenesis and differentiation				
Interleukin 3 receptor, α-chain	IL-3Rα	445664	5.32	1.23
Colony stimulating factor, macrophage	mCSF	634838	4.12	1.32
Glial cell line-derived neurotrophic factor	GNDF	425671	3.96	0.80
Integrin α-5 (fibronectin receptor)	Iα5	476908	3.55	0.94
Early growth response-1	EGR-1	608153	3.65	0.58
Jun oncogene	JUN	949554	3.01	1.11
Twist gene homolog	TWIST	479367	2.95	1.54
Forkhead homolog 14	FKH-14	541099	2.91	1.08
Wee 1-like protein kinase	Wee-1	539548	2.75	1.95
Insulin-like growth factor binding protein 10	IGF-BP10	557055	2.41	1.48
Sex-determining region Y (SRY)-box containing gene 2	SRY-2	351033	2.39	0.59
Interleukin 4 receptor alpha	IL-4Rα	721594	2.30	0.80
Mouse mRNA for dbpa murine homolog	DBPA	602275	2.29	1.65
Expressed sequence tags, moderately similar to MAK16 (*S. cerevisae*)	MAK16	537328	2.27	1.70
Ngfi-A binding protein 2	NGFI-A BP-2	476298	2.31	1.25
MAD (mothers against decapentaplegic) homolog 5 (*Drosophila*)	MAD5	551401	2.24	1.49
Early development regulator	EDR	616348	2.22	1.67
Ets variant gene 6 (TEL oncogene)	TEL	402134	2.21	0.97
Apoptosis				
Mus musculus TDAG51 (T-cell death-associated gene)	TDAG51	694076	9.00	1.52
Mus musculus Fas-binding protein (Daxx)	Daxx	736796	5.99	1.55
Cellular processes				
Murine mRNA for replacement variant histone H3.3	vH3.3	618380	3.30	1.39
Kinesin heavy chain member 1A	Kin1A	492514	2.83	0.67
Mouse chromatin nonhistone high-mobility group protein (HMG-I(Y))	HMG-1(Y)	616054	2.64	1.15

TABLE I
(continued)

	Symbol	Clone number	IGF-1	Insulin
Mus musculus mRNA for eRF1	eRF-1	572924	2.34	1.19
DEAD (aspartate-glutamate-alanine-aspartate) box polypeptide 5	DEAD5	537478	2.22	1.24
Splicing factor, arginine/serine 3 (SRp20)	SRp20	595904	2.41	1.69
Metabolism				
Murine Glvr-1 mRNA	GLVR-1	335579	4.88	1.20
Glycerol phosphate dehydrogenase 1, mitochondrial	GPDH	351221	2.74	0.91
Others				
Nuclear factor erythroid-derived 2, like 2	NF-E2	635541	2.90	0.86
Immediate early protein Gly96	Gly96	579574	2.46	1.13

[Adapted from Dupont J, Khan J, Qu BH, Metzler P, Helman L, LeRoith D 2001 Insulin and IGF-1 induce different patterns of gene expression in mouse fibroblast NIH-3T3 cells: identification by cDNA microarray analysis. Endocrinology 142:4969–4975.]

also showed that Twist is positively involved in the antiapoptotic effects of the IGF-1R. These studies show that a gene that is regulated by IGF-1 receptor activation may, in turn, regulate the function of the IGF-1 receptor function.

C. IGF-1-INDUCED GENES AND CANCER PROGRESSION

IGF-1-regulated genes were studied in a preneoplastic, immortalized breast cell line, 184htert. The advantage of utilizing this cell line is that genes may be identified that are affected by IGF-1 and the IGF-1 receptor signaling pathways at an early stage in the progression of cancer. These cells were created by using retroviral technology to introduce the human telomerase reverse transcriptase gene into normal breast epithelial cells. Of the $\approx$ 2000 known genes on the microarray chip, 156 (8%) were regulated by IGF-1. These genes exhibited various patterns of regulation; whereas some were either up- or downregulated at early time points, others were regulated in a biphasic manner. The IGF-1-responsive genes could be subdivided into various categories (e.g., transcription factors, cell cycle-related genes, genes involved in cancer progression, signaling-related genes, extracellular matrix genes, genes related to metabolism). Interestingly, IGF-1 regulated a large number of genes involved in angiogenesis. Many genes known to stimulate angiogenesis were upregulated

TABLE II
Genes That Are Specifically Upregulated by Insulin in NIH-3T3 Fibroblasts

	Symbol	Clone ID	IGF-1	Insulin
Morphogenesis and development				
Mouse alpha-B crystallin mRNA	CRYαB	605970	1.56	2.28
Calponin H1, smooth muscle	CNNh1	557012	1.27	2.10
Apoptosis				
Apoptotic protease activating factor 1	APAF-1	657503	1.33	2.20
Seven in absentia 1B	SIAH-1B	618379	1.30	2.04
Cellular processes				
Microtubule-associated protein tau	TAU	552102	1.36	2.23
Integrin alpha 6	Iα6	584662	1.63	2.05
Cytochrome P450 2d10			1.46	2.34
Others				
Prolactin receptor	PRL-R	520835	0.72	3.74
Delta-aminolevulinate dehydratase	DAH	518879	1.53	2.13
Expressed sequence tags, highly similar to envelope (ENV) polyprotein precursor		539102	1.79	2.13

[Adapted from Dupont J, Khan J, Qu BH, Metzler P, Helman L, LeRoith D 2001 Insulin and IGF-1 induce different patterns of gene expression in mouse fibroblast NIH-3T3 cells: identification by cDNA microarray analysis. Endocrinology 142:4969–4975.]

by IGF-1, whereas inhibitors of angiogenesis such as plasminogen activator inhibitor-1 (PAI-1) and metalloproteases were inhibited by IGF-1 (Table III). The effects of IGF-1 on many of these genes — including c-fos, VEGF, Fas ligand, cyp1A1, cyp1B1, interleukin-1β, and uPA — were validated by other techniques. Many of the genes that are regulated by IGF-1 are also responsive to the hypoxia-inducible factor-1alpha (HIF-1α) and cAMP response binding protein (CREB) transcription factors. Indeed, IGF-1 induced nuclear translocation of HIF-1α and the phosphorylated form of CREB, thereby inducing gene expression.

Thus, this study demonstrated that IGF-1 regulates the expression of many genes involved in cancer progression. This new information may be helpful when considering gene targeting for therapeutic uses in the treatment of cancer.

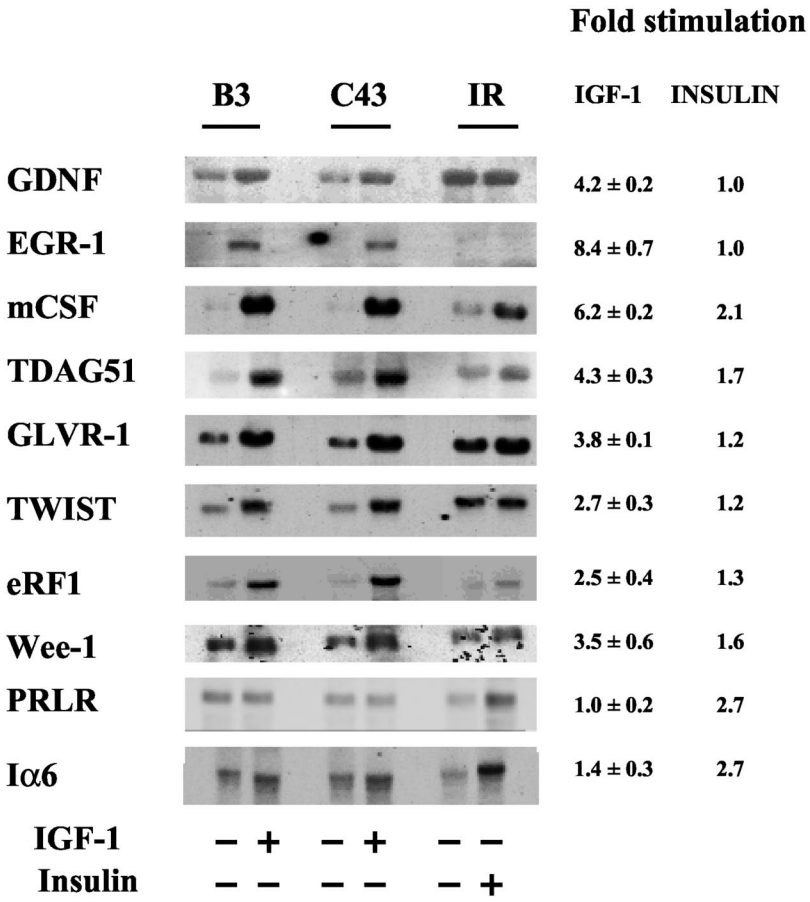

FIG. 3. Confirmation of the specific gene expression by IGF-1 or insulin using Northern blot analysis. Northern blot analysis was performed using RNA from cells expressing IGF-1 receptors (B3 and C43) or insulin receptors (IR), following stimulation. The specificity of stimulated gene expression correlated with the microarray results seen in Tables I and II. Abbreviations: CSF, colony-stimulating factor; EGR, early growth response; GDNF, glial cell line-derived neurotrophic factor; GLVR, gibbon ape leukemia virus receptor; PRLR, prolactin receptor; TDAG, T-cell death-associated gene. [Reprinted with permission from Dupont J, Khan J, Qu BH, Metzler P, Helman L, LeRoith D 2001 Insulin and IGF-1 induce different patterns of gene expression in mouse fibroblast NIH-3T3 cells: identification by cDNA microarray analysis. Endocrinology 142:4969–4975. Copyright The Endocrine Society.]

IV. Conclusion and Future Directions

The purpose of this review is to bring the possible applications of this exciting new technology to the attention of researchers. While cDNA microarray analysis is

TABLE III
Genes Specifically Affected by IGF-1 in Breast Epithelial Cells

B-cell lymphoma-2 interacting killer
c-fos
Cytochrome P450 1A1 and 1B1
Ferrodoxine reductase
GADD45 (growth arrest and DNA damage)
Interleukin 1 beta
Jun B
Low-density lipoprotein-related protein
Plasminogen activator inhibitor-1
Transferrin
Vascular endothelial growth factor

[Adapted from Oh JS, Kucab JE, Bushel PR, Martin K, Bennett L, Collins J, DiAugustine RP, Barrett JC, Afshari CA, Dunn SE 2002 Insulin-like growth factor-1 inscribes a gene expression profile for angiogenic factors and cancer progression in breast epithelial cells. Neoplasia 4:204–217.]

associated with a number of technical hurdles and experimental flaws, its utility has enormous potential, if used carefully, with multiple controls and constant validation. This approach may prove to be useful as a rapid screening test to identify the many genes that are differentially regulated in different tissues and systems. In particular, it may lead to the identification of genes not previously known to be affected by a particular process. Furthermore, in studying the progression of disease states and variations in gene expression, in various models, it may prove to be the more rapid and economical method. Numerous other applications undoubtedly will emerge as more investigators utilize this technology.

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