Insulin-like growth factor-I induces CLU expression through Twist1 to promote prostate cancer growth

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ABSTRACT

Clusterin (CLU) is cytoprotective molecular chaperone that is highly expressed in castrate-resistant prostate cancer (CRPC). CRPC is also characterized by increased insulin-like growth factor (IGF)-I responsiveness which induces prostate cancer survival and CLU expression. However, how IGF-I induces CLU expression and whether CLU is required for IGF-mediated growth signaling remain unknown. Here we show that IGF-I induced CLU via STAT3–Twist1 signaling pathway. In response to IGF-I, STAT3 was phosphorylated, translocated to the nucleus and bound to the Twist1 promoter to activate Twist1 transcription. In turn, Twist1 bound to E-boxes on the CLU promoter and activated CLU transcription. Inversely, we demonstrated that knocking down Twist1 abrogated IGF-I induced CLU expression, indicating that Twist1 mediated IGF-I-induced CLU expression. When Pten knockout mice were crossed with Lh/lt mice, the resultant IGF-I deficiency suppressed Twist1 as well as CLU gene expression in mouse prostate glands. Moreover, both Twist1 and CLU knockdown suppressed prostate cancer growth accelerated by IGF-I, suggesting the relevance of this signaling not only in an in vitro, but also in an in vivo. Collectively, this study indicates that IGF-I induces CLU expression through sequential activation of STAT3 and Twist1, and suggests that this signaling cascade plays a critical role in prostate cancer pathogenesis.

1. Introduction

Prostate cancer is the most common solid malignant tumor among males in Western countries (Jemal et al., 2010). A series of epidemiological and biological studies demonstrate that the insulin-like growth factor (IGF) axis is a critical regulator of growth, survival, and metastatic potential in a variety of malignancies and is closely implicated in prostatic carcinogenesis and prostate cancer progression as well as resistance to castration therapy (Chan et al., 1998; Krueckl et al., 2004; Nickerson et al., 2001; Wolk et al., 1998). We have previously demonstrated that IGF-I promotes human prostate cancer cell growth and that increased IGF-I receptor (IGF-IR) expression and signaling are components of castrate resistant progression (Krueckl et al., 2004; Takahara et al., 2011).

IGFs bind to the IGF-IR, which is a heterotetrameric type I receptor protein-tyrosine kinase composed of two ligand-binding α-subunits and two transmembrane β-subunits. The binding of ligand to IGF-IR induces auto-phosphorylation of the β-subunits of the receptor complex and further activation of the protein-tyrosine kinase activity (Hubbard et al., 1994; Weiss and Schlessinger, 1998). Once activated, IGF-IR recruits and phosphorylates various downstream targets such as the insulin receptor substrate-1 and -2 which activate many signaling pathways, including Ras/Raf/mitogen-activated protein kinase (MAPK) and PI3K/Akt, as well as signal transducer and activator of transcription 3 (STAT3; Zong et al., 2000) resulting in cell growth and survival.

Clusterin (CLU) is a stress-induced cytoprotective chaperone, and involved in many biological processes such as sperm maturation, tissue differentiation, tissue remodeling, membrane recycling, lipid transportation, cell proliferation and cell death. CLU has been shown expressed in many human cancers (Zhong et al., 2010). Increased levels of CLU have been reported in breast, colon, lung, bladder, prostate and other cancers (July et al., 2004; Kevans et al., 2009; Miyake et al., 2002; So et al., 2005; Steinberg et al., 1997). In prostate, CLU levels are low in benign prostate epithelial
cells, but increase in prostate cancers with higher Gleason grade (Steinberg et al., 1997). Furthermore, CLU expression increases as prostate cancers adapt to androgen-deprivation therapy (July et al., 2002). These data indicate that CLU is also implicated in prostate carcinogenesis and prostate cancer progression. Similarly, numerous evidences showed that a basic helix–loop–helix transcription factor Twist1 is also involved in pathogenesis of various cancers (Franco et al., 2011), including castration resistance in prostate cancer (Shiota et al., 2010).

IGF-I axis induced CLU expression after irradiation via Src-MEK-ERK1-ERG1 signaling in human breast cancer MCF-7 cells (Criswell et al., 2005). However, the mechanism and role of CLU induction by IGF-I in prostate cancer remain unrevealed. In this study, we set out to define links between IGF-I signaling and Twist1/CLU expression in prostate cancer, identifying STAT3 as a downstream effector of IGF-I.

2. Material and methods

2.1. Cell culture and transfection

The human prostate cancer cell line, PC-3, was purchased from the American Type Culture Collection (ATCC authentication by iso-enzymes analysis) and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Thermo Scientific, Waltham, MA, USA) supplemented with 5% fetal-bovine serum (FBS). The human prostate cancer cell line LNCaP was kindly provided by Dr. Leland W.K. Chung (Cedars-Sinai Medical Center, Los Angeles, CA, USA), tested and authenticated by whole-genome and whole-transcriptome sequencing on Illumina Genome Analyzer IIX platform in 2009. LNCaP cells were maintained in RPMI 1640 (Thermo Scientific) supplemented with 5% FBS.

2.2. Antibodies and reagents

Antibodies against Myc (sc-815), CLU (sc-6419) and Twist1 (sc-81417) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphorylated STAT3 Tyr705 (p-STAT3 Tyr705, #9131), anti-phosphorylated STAT3 Ser727 (p-STAT3 Ser727, #9134) and anti-STAT3 (#9139) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-Lamin B1 and anti-β-actin antibodies were purchased from Abcam (Cambridge, MA, USA) and Sigma (St Louis, MO, USA), respectively. Human recombinant IgG-I was obtained from Fitzgerald (Acton, MA, USA).

2.3. Plasmids and siRNAs

Twist1-Myc-Flag plasmid expressing C-terminally Myc-Flag-tagged Twist1 protein and the corresponding mock plasmid (Myc-Flag plasmid) were purchased from OriGene (Rockville, MD, USA). The Twist1 reporter plasmid (Twist–Luc) was kindly provided by Dr. Wang LH (Mount Sinai School of Medicine, New York, NY, USA; Cheng et al., 2008). CLU reporter plasmids (CLU–Luc –1998/+254, –1998/+702, –1116/+702, and –707/+254) containing various lengths of the promoter and first exon of the CLU gene were constructed as described previously (Shiota et al., 2010). The following double-stranded 25-bp siRNA oligonucleotides were commercially generated (Invitrogen, Carlsbad, CA, USA): 5'-CUUCCUGCGCUUCCAGUGUCUCGACGUCCG-3' for Twist1 siRNA #1; 5'-UUAGGGGCUGAACCUCUCCAGCUCCAGCUCCG-3' for Twist1 siRNA #2. The sequence of siRNA corresponding to the human CLU initiation site in exon II was 5'-GCCAGAGAUGCUUCAUCAGU-3' (Dharmacon Research Inc.). Stealth™ RNAi Negative Control Medium GC Duplex #2 (Invitrogen) was used as a control siRNA. Cells were transfected with the indicated siRNA or the indicated plasmid as previously described (Zoubeidi et al., 2010a; Zoubeidi et al., 2010b).

2.4. Quantitative reverse transcription (RT)–PCR

RNA extraction and RT–PCR were performed as described previously (Lamoureux et al., 2011). Real-time monitoring of PCR amplification of cDNA was performed using the following primer pairs and probes, Twist1 (Hs00361186 _m1), CLU (Hs00156548 _m1) and GAPDH (Hs03929097_g1) (Applied Biosystems) on ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) with TaqMan Gene Expression Master Mix (Applied Biosystems). Target gene expression was normalized to GAPDH levels in respective samples as an internal control. The results are representative of at least three independent experiments.

2.5. Western blot analysis

Whole-cell extracts were obtained by lysis of cells in an appropriate volume of ice-cold RIPA buffer composed of 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS) containing 1 mM Na3VO4, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail tablets (Complete, Roche Applied Science, Indianapolis, IN, USA). Nuclear and cytoplasmic extracts were obtained using Celllytic™ NuCLEAR™ Extraction Kit (Sigma) according to manufacturer’s protocol. Cellular extracts were clarified by centrifugation at 13,000×g for 10 min and protein concentrations of the extracts determined by a BCA protein assay kit (Thermo Scientific). Thirty micrograms of the extracts were boiled for 5 min in SDS sample buffer, separated by SDS–PAGE, and transferred onto a polyvinylidene difluoride (PVDF) membrane. Membranes were probed with dilutions of primary antibodies followed by incubation with horseradish peroxidase-conjugated secondary antibodies. After extensive washing, proteins were visualized by a chemiluminescent detection system (GE Healthcare, Buckinghamshire, UK).

2.6. Luciferase reporter assay

Prostate cancer cells were transfected with 0.5 μg of the indicated reporter plasmid, expression plasmid, siRNA, and 0.05 μg of pRL-TK as an internal control. 24 h post transfection, media was changed to serum-free media followed by IGF-I treatment. The luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega) and a microplate luminometer (EGG& Berthold). The Firefly luciferase activities were corrected by the corresponding Renilla luciferase activities and protein concentration. The results are representative of at least three independent experiments.

2.7. Chromatin immunoprecipitation assay (ChIP assay)

LNCaP cells were seeded and stimulated with 100 ng/ml IGF-I followed by paraformaldehyde cross-linking and micrococcal nuclease digestion to achieve a DNA smear of 200–1000 bp. ChIP assay on the indicated genes was performed using SimpleChIP™ Enzymatic Chromatin IP Kit (Agarose Beads) according to the manufacturer’s protocol (Cell Signaling Technology). Quantitative RT–PCR assay was performed using ABI PRISM 7900 HT Sequence Detection System with 2 μl of 20 μl DNA extraction, the primer pairs below and RT² Real-Time™ SYBR Green/Rox PCR master mix (Qiagen, Valencia, CA, USA). The results are representative of at least three independent experiments. The primer pairs for Twist1 promoter were Fw: 5'-TGCTTCTCCCATGACTG-3' and Rv: 5'-GAGTTCCAAAGGCCAACC-3' as described previously (Cheng
et al., 2008). The primer pairs for CLU – 02 (GPH025704(−)02A) targeting around –1403 bp, CLU – 01 (GPH025704(−)01A) targeting around –417 bp, CLU +07 (GPH025704(+)07A) targeting around +6603 bp, and RPL30 gene (exon 3) were described previously (Shiota et al., 2011).

2.8. Production and characterization of Ghrhr(lit/lit)/Cre/PTEN(fl/fl)

The production of Ghrhr(lit/lit)/Cre/PTEN(fl/fl) was previously described (Takahara et al., 2013). Briefly, we crossed pbARR2-Cre, PTEN (fl/fl) mice (Wang et al., 2003) with GHRHR (lit/lit) mice (Yang et al., 1996) to produce lit/lit and lit/+ PTEN −/− mice. Mice sera and prostates were harvested in accordance with the guidelines of the Canadian Council on Animal Care and with appropriate institutional certification between 15 and 20 weeks of age. Nineteen mice were collected for each cohort after genotyping to clarify lit heterozygosity (lit/+)) and homozygosity (lit/lit), Cre recombinase and PTEN fl/fl status from tail clip DNA. Serum IGF-I knockdown lit/+ mice at 15 and 20 weeks of age were obtained in accordance with the guidelines of the Canadian Council on Animal Care and with appropriate institutional certification. Immunohistochemical staining was conducted as previously described (Zoubeidi et al., 2010b) using the Ventana Discover XT™ autostainer (Ventana Medical System, Tuscan, AZ, USA) with enzyme labeled biotin streptavidin system and solvent resistant DAB Map kit by antibodies against CLU (Santa Cruz Biotechnology), IGF-IR (Sigma), p-STAT3 Tyr705 (Cell Signaling Technology), Twist1 (Sigma).

2.11. Statistical analysis

All data were assessed using the Student’s t-test. Levels of statistical significance were set at $P < 0.05$.

3. Results

3.1. IGF-I induces both Twist1 and CLU expression in prostate cancer cells

It is known that IGF-I activates Twist1 in NIH-3T3 fibroblasts (Dupont et al., 2001) and CLU expression in breast cancer cell lines (Criswell et al., 2005). Since Twist1 is a transcription factor, we intended to determine whether IGF-I induced CLU expression via Twist1 in human prostate cancer cells. First, we examined the appropriate concentration inducing Twist1 and CLU expression in LNCaP and PC-3 cells. We found that 100ng/ml of IGF-1 is the optimal concentration inducing CLU and Twist expression both in LNCaP and PC-3 cells (data not shown). Then, we chose 100 ng/ml IGF-I for treatment thereafter. Next, LNCaP and PC-3 cell lines were treated with IGF-I in a time dependent manner and both Twist1 and CLU expression were evaluated at mRNA and protein levels. We found that IGF-I increased Twist1 as well as CLU expression at transcript level (Fig. 1A) and protein level (Fig. 1B) in LNCaP and PC-3 cells.

3.2. Twist1 binds to CLU promoter region and regulates CLU expression

The finding above prompted us to examine the functional link between Twist1 transcription factor and CLU. As shown in Fig. 2A, Twist1 knockdown using 2 different Twist1-specific siRNAs...
Fig. 2. Twist1 binds to CLU promoter region and regulates CLU expression. (A) LNCaP cells were transfected with 40 nM of the indicated siRNA. At 72 h after transfection, quantitative RT–PCR was performed using the primers and probes for Twist1, CLU and GAPDH. Each transcript level from cells transfected with control siRNA was set as 1. Boxes, mean; bars, ±s.d. *P < 0.05 (compared with control siRNA). Whole-cell extracts were analyzed by SDS–PAGE and western blot analysis with specific antibodies. (B) LNCaP cells were transfected with 1.0 μg/mL of the indicated expression plasmid. At 72 h after transfection, quantitative RT–PCR was performed using the primers and probes for CLU and GAPDH. Each transcript level from mock-transfected cells was set as 1. Boxes, mean; bars, ±s.d.*

Since Twist1 is known as an E-box (5′-CANNTG-3′) binding transcription factor (Li et al., 1995), we investigated whether Twist1 directly regulates CLU transcription. We first searched for reduced basal CLU expression at both transcript and protein levels in LNCaP cells. Inversely, Twist1 over-expression up-regulated CLU mRNA and protein expression (Fig. 2B).
putative E-box binding sites in the CLU promoter region between –2000 bp and +500 bp from transcription start site (TSS) and found that are 10 E-boxes in CLU promoter as shown in Fig. 2C. We next analyzed CLU promoter activity using different truncated regions (Fig. 2C). We found that CLU promoter activity was highest between –1998 bp and –702 bp in LNCaP cells which contains 7 E-boxes (Fig. 2D) as a potential Twist1-binding sites, similarly to the result using PC-3 cells (Shiota et al., 2012). To further evaluate if Twist1 can regulates CLU promoter activity, Twist1 was overexpressed and CLU transcriptional activity was analyzed using different CLU promoter constructs. As shown in Fig. 2E, Twist1 increased luciferase activity of CLU–Luc –1998/-702, but not CLU–Luc –1116/-702. These findings suggested that the cis-element of CLU promoter region containing –1998/-1116 bp was activated by Twist1. Inversely, Twist1 knockdown reduced CLU promoter transcriptional activity (Fig. 2F). To confirm Twist1 binding to CLU promoter region, we performed ChIP assay against CLU gene in LNCaP cells. The results showed that Twist1 bound to CLU

Fig. 3. IGF-I induces CLU expression via Twist1. (A) LNCaP (left) and PC-3 (right) cells were transfected with 40 nM of the indicated siRNA and incubated for 48 h, and then cells were treated with 100 ng/mL IGF-I. After 1.5 h (Twist1) or 12 h (CLU), quantitative RT–PCR was performed using the primer pairs and probes for Twist1, CLU and GAPDH. Each transcript level from non-treated cells was set as 1. Boxes, mean; bars, ±s.d.* P < 0.05 (compared with no treatment). (B) LNCaP (left) and PC-3 (right) cells were transfected with 40 nM of the indicated siRNA and 0.05 lL/mL of pRL-TK, incubated for 24 h, and then cells were and treated with IGF-I. After 24 h, the luciferase activity of CLU–Luc transfected with control siRNA without IGF-I was set as 1. Boxes, mean; bars, ±s.d.* P < 0.05 (compared with no treatment).
promoter regions around -1400 bp from TSS represented by CLU -02, while they did not bind to the CLU gene regions around +6600 bp from TSS and RPL30 gene (Fig. 2G).

3.3. IGF-I induces CLU expression via Twist1

To further evaluate whether Twist1 expression is required for IGF-I-induced CLU expression, Twist1 was silenced and levels of Twist1 and CLU were evaluated in the absence or presence of IGF-I. Twist1-specific siRNA successfully down-regulated both basal and IGF-I-induced Twist1 expression (Fig. 3A, left) as well as CLU mRNA at basal level. Interestingly, we found that Twist1 knockdown abrogated IGF-I-induced CLU expression at mRNA levels (Fig. 3A, right) and protein levels (Fig. 3B) in both LNCaP and PC-3 cells. These data suggest that Twist1 is involved in basal CLU expression, as well as required for IGF-I-induced CLU expression. In addition, luciferase reporter assay using CLU reporter plasmid also revealed that Twist1 knockdown ameliorated CLU induction by IGF-I (Fig. 3C).

3.4. IGF-I activates STAT3 transcription factor, resulting in Twist1 up-regulation

To investigate the mechanism of Twist1/CLU induction by IGF-I, we focused on STAT3 transcription factor because it was reported that STAT3 transcriptionally regulated Twist1 expression (Cheng et al., 2008). We next analyzed the effect of IGF-I on STAT3 phosphorylation. Our data showed that IGF-I induces STAT3 phosphorylation only on Tyr705 but not on Ser727, resulting in an activation of STAT3 (Fig. 4A). This result was supported by the finding that IGF-I facilitated STAT3 translocation into nucleus (Fig. 4B). As a result, IGF-I stimulated binding to Twist1-promoter region, resulting in activation of Twist1 transcription (Fig. 4C), thereby augmented Twist1 binding to CLU-promoter region (Fig. 4D). Consistently, reporter assay using Twist1 reporter plasmid showed an increased transcription of Twist1 gene by IGF-I (Fig. 4E). These data show that IGF-I promoted STAT3 activation and STAT3 is at least in part needed to mediate the IGF-I-induced Twist1 expression and subsequent CLU expression.

3.5. IGF-I/Twist1/CLU signaling plays a critical role in mice prostate cancer proliferation

To investigate the biologic relevance of the above findings, we assessed Twist1 and CLU expression in prostate tissues from PTEN knockout mice (Wang et al., 2003) crossed with litter/litter mice, which harbor growth-hormone-releasing hormone receptor (GHRHR) mutation abolishing GHRHR function (Wang et al., 2003). Lack of growth-hormone-releasing hormone signaling in litter/litter mice results in marked reduction of serum growth hormone, which in turn leads to reducing serum IGF-I level (Fig. 5A), known to correlate with IGF-I level in prostate (Wang et al., 2008). Consistently with the preceding in vitro data, Twist1 and CLU expression were lower in prostate tissues, harvested between 15 and 20 weeks of age, from PTEN knockout litter/litter mice compared with those of PTEN knockout litter/+ mice (Fig. 5B). As well, immunohistochemistry against prostate tissues from PTEN knockout litter/litter mice and PTEN knockout litter/+ mice.
knockout lit/+ mice suggested that prominent decreased levels of IGF-IR and Twist1 in lit/lit mice (Fig. 5C).

Previously we showed that prostate cancer grew less rapidly in lit/lit mice compared with lit/+ mice in an in vitro as well as an in vivo (Takahara et al., 2011). Subsequently, we examined whether cell proliferation induced by IGF-I is affected by Twist1 or CLU silencing. As we previously reported, LNCaP cell growth was promoted by IGF-I, while this growth promotion was almost completely abolished by either Twist1 or CLU knockdown (Fig. 6A), suggesting that both Twist1 and CLU are important downstream mediators of IGF-I-induced prostate cancer growth.

4. Discussion

In this study, we identified a novel mechanism by which IGF-I regulates CLU expression in prostate cancer cells via the STAT3–Twist1 pathway. We went onto demonstrate that once IGF-I activates STAT3, STAT3 translocates to the nucleus, binds to the Twist1 promoter, resulting in Twist1 up-regulation. Twist1 subsequently binds to E-boxes on CLU promoter and enhance CLU expression, thereby creating a feed-forward loop which leads to increase of cell proliferation in prostate cancer (Fig. 6B).

Our data showed that IGF-I induced STAT3 phosphorylation in prostate cancer cells, confirming previous reports in human fibroblasts (HEK293T cells; Zong et al., 2000) and suggesting that the role of IGF-I on STAT3 activation was conserved across different cell lines. Once phosphorylated, STAT3 translocated to the nucleus and regulated Twist1 expression in breast cancer cells (Cheng et al., 2008). STAT3 activation positively correlated with Twist1 expression in breast cancer tissues (Cheng et al., 2008). Similarly, epidermal-growth factor (EGF)-induced Twist1 transcription was reported to be mediated by STAT3 in several cancer cells (Lo et al., 2007). Moreover, in hepatocellular carcinoma,
activated STAT3 and Twist1 expressions were positively correlated (Zhang et al., 2012). Thus, the connection between STAT3 and Twist1 has been established in various cancers including prostate cancer (Cheng et al., 2008; Cho et al., 2013; Hsu et al., 2012; Teng et al., 2013). Additionally, in this study, STAT3 phosphorylation at Tyr705 was induced by IGF-I concurrent with nuclear translocation, which is consistent with the previous report (Wen et al., 1995). Moreover, we found that STAT3 bound to Twist1 promoter region in prostate cancer, leading to Twist1 gene expression, which was increased by IGF-I treatment.

Twist1, a basic helix–loop–helix transcription factor, has been described as a proto-oncogene (Hamamori et al., 1997; Quertermous et al., 1994) that promoted breast cancer metastasis (Yang et al., 2004). Similar to CLU, Twist1 was also up-regulated in various malignant tumors, including prostate cancer (Wallerand et al., 2010; Wang et al., 2004). Moreover, we have recently shown that Twist1 was involved in prostate cancer growth (Shiota et al., 2008) as well as resistance to castration through androgen receptor (Shiota et al., 2010). Collectively, Twist1 plays a key role in the development and progression of prostate cancer similar to that ascribed to CLU. Like Twist1 (Dupont et al., 2001), CLU was also known to be induced by IGF-I (Criswell et al., 2005). In this study, Twist1 knockdown decreased basal CLU transcript and protein, as well as IGF-I-induced CLU expression, indicating that IGF-I-induced CLU expression was mediated by Twist1. Furthermore, we found that Twist1 regulated CLU expression by reporter assay and ChIP assay. These findings link Twist1 regulation of CLU expression, under IGF-I stimulation, as a potential pathway that promotes prostate cancer growth.

The pbARR2-Cre, PTEN+/− mice model, which lead to de novo formation of prostate tumors, is one model that mimics human prostate cancer from initiation to local invasion and metastasis (Trotman et al., 2003; Wang et al., 2003). Prostate-specific loss of PTEN expression resulted in invasive carcinoma with lymphovascular invasion within 12 weeks, which progressed to lung metastasis (Wang et al., 2003). Deletion or mutation of the tumor suppressor PTEN gene has been implicated in many human cancers and has been seen in up to 30% of primary prostate cancers and >64% of prostate metastases, making PTEN an important candidate gene for prostate cancer development and progression (Majumder and Sellers, 2005; Suzuki et al., 1998). Furthermore, CLU expression was elevated in PTEN knockout mice (Wang et al., 2003). To define links between IGF-I signaling and CLU in prostate cancer growth, we crossed pbARR2-Cre, PTEN+/− mice with GHRHR (lit/lit) mice. It has been known that in lit/lit mice, several proto-oncogenic pathways including MAPK and PI3K/Akt were down-regulated (Takahara et al., 2011). In addition, in PTEN knockout lit/lit mice model, Twist1 as well as CLU expression was reduced in IGF-I-deficient lit/lit mice, which supported our in vitro data that IGF-I-induced Twist1 and CLU expression in LNCaP and PC-3 cells. Furthermore, this study suggested that Twist1 as well as CLU plays key roles in IGF-I-induced prostate cancer cell proliferation. Since IGF-I has been a well-known promoter of prostate cancer growth, these data identified Twist1 and CLU as important mediators of IGF-I-stimulated prostate pathogenesis in this model.

In summary, we identified a novel Twist1/CLU pathway stimulated by IGF-I involving STAT3 phosphorylation, and then CLU. Therefore, signaling from IGF-I to CLU provided a molecular mechanism that might explain at least in part the influence of IGF-I on prostate cancer.

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References


