Human Krüppel-like factor 11 differentially regulates human insulin promoter activity in β-cells and non-β-cells via p300 and PDX1 through the regulatory sites A3 and CACCC box

PERAKAKIS, Nikolaos, et al.

Abstract

Human Krüppel-like factor 11 (hKLF11) has been characterised to both activate and inhibit human insulin promoter (hInsP) activity. Since KLF11 is capable to differentially regulate genes dependent on recruited cofactors, we investigated the effects of hKLF11 on cotransfected hInsP in both β-cells and non-β-cells. hKLF11 protein interacts with hp300 but not with hPDX1. Overexpressed hKLF11 stimulates PDX1-transactivation of hInsP in HEK293 non-β-cells, but confers inhibition in INS-1E β-cells. Both hKLF11 functions can be neutralised by the p300 inhibitor E1A, increased hp300 levels (INS-1E), dominant negative (DN)-PDX1 and by mutation of the PDX1 binding site A3 or the CACCC box. In summary, hKLF11 differentially regulates hInsP activity depending on the molecular context via modulation of p300:PDX1 interactions with the A3 element and CACCC box. We postulate that KLF11 has a role in fine-tuning insulin transcription in certain cellular situations rather than representing a major transcriptional activator or repressor of the insulin gene.

Reference


DOI : 10.1016/j.mce.2012.07.003
PMID : 22801105
Human Krüppel-like factor 11 differentially regulates human insulin promoter activity in β-cells and non-β-cells via p300 and PDX1 through the regulatory sites A3 and CACCC box

Nikolaos Perakakis, Despoina Danassi, Marcus Alt, Eleni Tsaroucha, Amir E. Mehana, Natalie Rimmer, Katharina Laubner, Haiyan Wang, Claes B. Wollheim, Jochen Seufert, Günter Päth

Abstract

Human Krüppel-like factor 11 (hKLF11) has been characterised as both activate and inhibit human insulin promoter (hInsP) activity. Since KLF11 is capable to differentially regulate genes dependent on recruited cofactors, we investigated the effects of hKLF11 on cotransfected hInsP in both β-cells and non-β-cells. hKLF11 protein interacts with p300 but not with hPDX1. Overexpressed hKLF11 stimulates PDX1-transactivation of hInsP in HEK293 non-β-cells, but confers inhibition in INS-1E β-cells. Both hKLF11 functions can be neutralised by the p300 inhibitor E1A, increased hp300 levels (INS-1E), dominant negative (DN)-PDX1 and by mutation of the PDX1 binding site A3 or the CACCC box. In summary, hKLF11 differentially regulates hInsP activity depending on the molecular context via modulation of p300/PDX1 interactions with the A3 element and CACCC box. We postulate that KLF11 has a role in fine-tuning insulin transcription in certain cellular situations rather than representing a major transcriptional activator or repressor of the insulin gene.

1. Introduction

Krüppel-like factor (KLF)11 is a member of the Sp1/KLF transcription factor family which shares binding to GC-rich or CACCC sequences via three conserved C-terminal zinc-finger domains (McConnell and Yang, 2010). KLF proteins are ubiquitously expressed throughout mammalian tissues and participate in virtually all aspects of cellular function as transcriptional repressors or activators depending on the molecular context (Kaczynski et al., 2003; Ou et al., 2004). Among KLFs, KLF10 and KLF11 form a subgroup defined by TGF-β-inducible expression and were therefore alternatively named TGF-β-inducible early response genes (TIEG)1 and 2, respectively (Cook et al., 1998; Subramaniam et al., 1995).

KLF11 is enriched in muscle and pancreas (Cook et al., 1998) and published results characterised KLF11 as a negative regulator of exocrine pancreatic cell proliferation in transgenic mice with acinar cell-specific KLF11 overproduction (Fernandez-Zapico et al., 2003). Within the endocrine pancreas, KLF11 was initially characterised as an activator of the human insulin gene promoter (hInsP) (Neve et al., 2005). This study further reported an association between KLF11 gene variants and early-onset diabetes as well as common type 2 diabetes in French and Northern European populations, respectively. Based on these associations, KLF11 gene variants which failed to activate the insulin promoter were proposed to represent a new MODY (MODY7) (Fernandez-Zapico et al., 2009). However, associations between KLF11 gene/promoter variants and MODY or type 2 diabetes could not be confirmed in other populations including full-heritage Pima Indians (Ma et al., 2008), Danish (Florez et al., 2006; Gutierrez-Aguilar et al., 2008) and Japanese individuals (Kuroda et al., 2009; Tanahashi et al., 2008).
Nevertheless, a significant association between KLF11 gene/pro- moter variants and reduced insulin sensitivity in glucose tolerant Danish subjects was verified (Gutierrez-Aguilar et al., 2008). Vari- ability of these results may be at least in part explainable by the close structural and functional relationship of KLF11 to KLF10 (Subramaniam et al., 2007). As an example for shared function among KLFs, coexpressed KLF10 as well as KLF13, KLF14 and KLF16 have recently been demonstrated to activate the hInsP (Bon- nefond et al., 2011).

The initial report by Neve et al. (2005) and the recent study of Bonnefond et al. (2011) characterise KLF11 as a stimulator of cotransfected hInsP reporter in β-cell lines. Our own research, however, could not confirm these findings. We observed that hKLF11 inhibits hInsP in rat INS-1E as well as mouse β-TC3 β-cells (Niu et al., 2007). A negative regulation of hInsP by cotransfected hKLF11 was also observed by others in mouse MIN6-m9 β-cells (Kuroda et al., 2009). These opposite findings raise the question whether the employed experimental settings reflect differential actions of KLF11 that depend on the exact molecular context as it has been reported earlier for the human monoamine oxidase gene (MAO) B gene (Ou et al., 2004). Differential regulation of gene expression is a general feature in several other KLFs because of their ability to recruit transcriptional corepressors as well as coac- tivators in distinct cellular situations (Kaczynski et al., 2003; McConn and Yang, 2010). Alternatively, KLF11 may influence hInsP indirectly since it can bind the conserved area II region within the pancreatic duodenal homeobox protein (PDX)1 gene pro- moter and is potentially able to stimulate expression of PDX1 which is a major transactivator of insulin gene expression (Fernandez-Zapico et al., 2009). However, PDX1 function strictly depends on the transcriptional cofactor p300, and KLF11 has been demon- strated to interact with p300 (Fernandez-Zapico et al., 2009). The importance of p300 is underscored by a reduction of KLF11-mediated hInsP activation if a mutant dominant negative p300 is coex- pressed (Bonnefond et al., 2011).

Taken together, there is consensus that KLF11 can regulate hInsP. The described conflicting findings on stimulation versus inhibition indicate that the exact molecular mechanism is not yet fully understood. To characterise context-dependent regulation of the hInsP by KLF11, we analysed the contributing roles of the p300/PDX1 transcriptional complex and relevant promoter re- sponse elements in both β-cells and non-β-cells.

2. Methods

2.1. Cell culture

Rat INS-1E β-cells and INS-1-derived transgenic INSrβ β-cell lines were routinely cultured in RPMI 1640 (11.1 mM glucose) containing 10% FBS, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate and 50 μM β-mercaptoethanol. INSrβ β-cell lines enable doxycycline (Dox)-inducible overexpression of either mouse Pdx1 or its mutated dominant negative (DN)-Pdx1 variant lacking the complete N-terminal transacti- vation domain (the first 79 amino acids). INSrβ β-cell lines were generated and characterised as described earlier (Wang et al., 2001). Human embryonic kidney 293 cells (HEK293) were cultured in DMEM (25 mM glucose) containing 10% FBS, 2 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.2. Plasmid construction

Construction of human KLF11 expression plasmid (hKLF11- pcDNA 3.1) and human insulin promoter-driven secreted alkaline phosphatase (SEAP) reporter plasmids (5'-deletion series –881 + 54 to –101 + 54hInsP-pSEAP) has been described previ- ously (Niu et al., 2007). The human p300 coding sequence was sub- cloned from CMV6-p300 (kindly provided by Timothy J. Kieffer, University of British Columbia Vancouver, BC Canada) into the CMV promoter-driven pcDNA3.1 (Invitrogen/Life Technologies GmbH, Darmstadt, Germany) to obtain the hp300-pcDNA3.1 expression plasmid. The human PDX1 coding sequence was sub- cloned from hPDX1-pCMV5 (kindly provided by Doris A. Stoffers, University of Pennsylvania, School of Medicine, Philadelphia, PA, USA) into pcDNA3.1 to generate the hpPDX1-pcDNA3.1 expression plasmid. The phβ9Q-E1A plasmid (Bannister and Kouzarides, 1995) expresses E1A under the control of the Rous sarcoma virus (RSV) promoter (kindly provided by Bernd Groner, Georg-Speyer- Haus, Institute for Biomedical Research, Frankfurt am Main, Germany). A1, A3 and G22 box of –881 + 54hInsP promoter sequence were mutated as described earlier (Le Lay and Stein, 2006). The CACC box within a –387 + 54hInsP promoter fragment was muta- tated from CCCCACCCC to CCGGATTCC as described earlier (Niu et al., 2007). All mutations were performed using the Quick-Change Site-Directed Mutagenesis Kit (Stratagene, Heidelberg, Germany). Potential protein binding between hKLF11, hpPDX1 and hp300 was analysed by using the CheckMate™ Mammalian Two- Hybrid System (Promega GmbH, Mannheim, Germany). hKLF11, hpPDX1 and hp300 coding sequences were subcloned from above described plasmids into multiple cloning regions of each of the as- say-provided expression plasmids pACT (expresses VP16 fusion proteins) and pBIND (expresses GAL4 fusion protein). Binding of pACT and pBIND expressed fusion proteins activates luciferase expres- sion by the pG5lac reporter plasmid. Accuracy of all se- quences and mutations was checked by sequencing.

2.3. Protein extraction and Western blot analysis

HEK293, β-TC3 and INS1E cells were seeded in 100 mm dishes and grown to optical confluency. Whole cell extracts were gener- ated by using RIPA buffer (Upstate/Biomial, Hamburg, Germany) containing 1 tablet/10 ml Complete Mini EDTA-free protease inhibi- tor cocktail (Roche, Mannheim, Germany). Nuclear extracts were generated as described earlier (Schreiber et al., 1989). Three mil- lion INS-ββ β-cells were seeded in 60 or 100 mm dishes. After two days day ectopic PDX1 or DN-PDX1 expression was induced by addition of Dox. Whole cell extracts were generated 24 h post addition of Dox as explained above. In general, 7 μg extracted pro- teins per sample were separated on 10% polyacrylamide SDS gels and transferred to PVDF membranes by semi-dry blotting using Towbin transfer buffer. KLF11, actin and PDX1 were detected by their respective goat polyclonal antibodies (Thermo Scientific, CA) and pACT or pBIND expressed fusion proteins activates luciferase expres- sion by the pG5lac reporter plasmid. Accuracy of all se- quences and mutations was checked by sequencing.

2.4. SEAP reporter gene experiments

INS-1E β-cells or HEK293 cells were seeded in 6-well culture plates at a density of 300,000 cells/well in their respective med- ium. Next day cells were transiently transfected using Metafectene Pro (Biontex, Martinsried/Planegg, Germany) using a ratio of 2 μl Metafectene Pro per 1 μg DNA. After 48 h supernatants were collected for measurement of SEAP using the BD Great EscAPe SEAP Chemiluminescence Detection Kit (BD Biosciences Clontech, Heidelberg, Germany). INSrβ-pPDX1 and INSrβ-DN-PDX1 β-cells were seeded 300,000 cells/well each in 6-well plates. Next day ectopic gene expression was induced by addition of 500 ng/ml Dox
According to Wang et al. (2001), cells were transiently transfected at day 3 and supernatants were collected at day 7 after seeding. Generally, total amount of transfected DNA was adjusted to 1 μg by addition of pcDNA3.1 empty vector. In exception, total transfected DNA was 1.25 μg in experiments shown in Fig. 1B and 1.5 μg in those for Fig. 3A.

2.5. Analysis of protein interaction

We employed the Check Mate Mammalian Two-Hybrid assay consisting of three plasmids as described above in Plasmid construction. HEK293 cells were seeded in 24-well plates at a density of 50,000 and directly transfected in parallel with pACT-(X), pBIND-(Y) and pG5luc (0.33 μg each) by using Metaffectene Pro as described above. Transfected DNA amount was 1 μg in all experimental groups. X and Y refer to coding sequences of two respective proteins tested for interaction as indicated in Fig. 6. Forty eight hours after transfection cells were harvested for analysis of luciferase signals by using the Dual-Luciferase Reporter Assay System (Promega GmbH) according to the manufacturer’s protocol.

2.6. Statistical analysis

Results were analysed for statistical differences by t test (comparison between two groups) or ANOVA followed by Bonferroni post hoc test (three and more groups) using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. hKLF11 interacts with hp300 and synergistically stimulates p300/PDX1-induced hInsP activity in non-β-cells

In our initial report, we postulated that KLF11 may interact with p300 or PDX1. Therefore, we analysed potential protein interactions by a mammalian-two hybrid system. HEK293 cells were transiently cotransfected with a pG5luc reporter plasmid as well as pACT and pBIND expression plasmids containing coding sequences of proteins as indicated in the figure. pACT/pBIND-hKLF11 and pACT/pBIND-hPDX1 signals define unspecific signal background and the known interaction between hp300 and hPDX1 served as positive control. Protein interaction was observed between hKLF11 and hp300 but not between hKLF11 and hPDX1. Means ± SD, n = 4; *p < 0.05 vs. individual control. (B) HEK293 cells were transiently cotransfected with -884 + 54hInsP reporter plasmid, hp300, hPDX1 and hKLF11 or pcDNA3.1 empty vector (mock). Compared to mock, overexpression of hKLF11 alone or in combination with hp300 did not affect hInsP but hKLF11 significantly enhanced hPDX1- and hp300/hPDX1-induced promoter activation. Additional cotransfection of the p300/PDX1 inhibitor E1A completely abolished hInsP activation. Means ± SD, n = 3; ***p < 0.01 and ****p < 0.001 vs. individual mock; $p < 0.05 vs. hKLF11 and hPDX1. (C) Detection of KLF11 protein in whole cell extracts (WCE) and nuclear extracts (NE) of HEK293, β-TC3 and INS-1E cells. Representative blot, n = 3.

To further explore the underlying molecular mechanism, we evaluated hKLF11 effects on hInsP in HEK293 cells (Fig. 1B). Cells were transiently cotransfected with –884 + 54hInsP reporter plasmid, hp300, hPDX1 and hKLF11 and E1A (0.25 μg each). hKLF11 was absent in individual mock controls. Activity of hInsP in the absence of the β-cell-specific transcription machinery was very low and defines the background which was not altered by further coexpression of hKLF11. Overexpression of hp300 alone or in combination with hKLF11 did not affect hInsP activity in HEK293 cells. Substantial hInsP activation could be induced by coexpression of hPDX1 alone (endogenous p300 is present in HEK293 cells) and was much more pronounced by the combination of hPDX1 and hp300. This was an expected result since PDX1 is a β-cell-specific PDX1 is absent in non-β-cells and the concentration of p300 is known to be the limiting factor of PDX1 action (Stanojevic et al., 2004). Interestingly, hKLF11 synergistically stimulated hPDX1- and hp300/PDX1-induced hInsP activity in HEK293 cells. This finding contrasts with our earlier observed KLF11-mediated inhibition of hInsP activity in β-cell lines (Niu et al., 2007). In some experiments, we further
coexpressed the established p300/PDX1 inhibitor E1A (Qiu et al., 2002) which completely abrogated hInsP activity. This observation confirms that p300 and (ectopic) PDX1 are strictly required for the stimulatory impact of KLF11 on hInsP in HEK293 non-β-cells.

In addition, we have verified endogenous KLF11 protein expression in HEK293 whole cell extracts and for control also in β-TC3 nuclear extracts and INS-1E whole cell extracts (Fig. 1C). Endogenous KLF11 expression in both β-cell lines confirms our previous findings (Niu et al., 2007).

3.2. Inhibition of hInsP by KLF11 in β-cells depends on p300 and PDX1

To investigate the role of p300 for the previously reported inhibition of hInsP in INS-1E and β-TC3 β-cells (Niu et al., 2007), we transiently transfected INS-1E with the −884 + 54hInsP reporter plasmid, hKLF11 (both 0.25 µg) and different amounts of hp300. Mock controls lack hKLF11. Significant reduction of hInsP activity by coexpressed hKLF11 was observed as reported earlier (Fig. 2A). Interestingly, additional coexpression of 0.1 or 0.5 µg hp300 completely abolished the inhibitory impact of hKLF11 indicating that its inhibitory function can be blocked by excessive concentrations of hp300 protein.

To evaluate the specific role of PDX1 in this context, we employed two INSr β-cell lines with Dox-inducible overexpression of either mouse Pdx1 (r-D-PDX1) or its dominant negative variant (DN)-PDX1 that lacks the complete transactivation domain (r-DN-Pdx1). Dox-induced ectopic protein production was analysed by Western blotting and found maximal at 500 ng/ml Dox (Fig. 2B). In whole cell extracts, we observed in both INSr β-cell lines a signal at 31 kDa. These signals indicated by asterisks likely represent the cytosolic 31 kDa variant of PDX1 described by Macfarlane et al. (1999) and served as loading control. Note that ectopic DN-PDX1 expression is slightly leaky (band at 0 mM Dox). Consequently, expansion of INSr-DN-Pdx1 β-cells is slower than in INSr-Pdx1.

INSr-PDX1 and INSr-DN-PDX1 β-cells with induced ectopic protein expression were transiently cotransfected with −884 + 54hInsP reporter plasmid and hKLF11 (0.5 µg each). Mock controls lack hKLF11. As observed before in INS-1E β-cells (Fig. 2A), overexpression of hKLF11 substantially inhibited hInsP activity in both INSr-PDX1 and INSr-DN-PDX1 β-cells (Fig. 2C). The inhibitory effect of hKLF11 was not altered by induced ectopic PDX1 but was almost completely diminished by induced ectopic DN-PDX1 expression. Note that Fig. 2C does not reflect DN-PDX1-mediated general reduction of hInsP transactivation since values were normalised to individual mock level.

These results demonstrate that hKLF11-mediated inhibition of hInsP in β-cells is mediated via PDX1. Promoter repression by hKLF11 can be blocked by excessive free hp300 indicating a situation where hp300 molecules neutralise hKLF11 and remaining free hp300 molecules still support the formation of functional PDX1 transcriptional complexes.

3.3. Effects of hInsP S-deletion and mutation of PDX1 binding sites or the CACCC box on hPDX1 and hKLF11-induced promoter activity in non-β-cells

To locate the promoter region responsible for synergistic enhancement of PDX1-mediated hInsP activation by hKLF11, we investigated a series of hInsP S-deletion constructs in HEK293 cells (Fig. 3A). The −881 + 54hInsP full length fragment contains the PDX1 binding elements A5 (−319 to −307), A3 (−216 to −207), GC2 (−145 to −140) and A1 (−83 to −75) which were deleted from S in a stepwise approach. HEK293 cells were transiently transfected with −881 + 54hInsP or its S-deletion constructs, hPDX1 and hKLF11 (0.5 µg each plasmid). Individual mock controls

![Fig. 2. KLF11-mediated inhibition of hInsP in β-cells depends on p300 and PDX1.](http://dx.doi.org/10.1016/j.mce.2012.07.003)
and KLFL1 decreased with shortening of promoter and was abolished at 3.7fold). The very short -101 fragment displayed general reduced activity. hPDX1-no impact on promoter activity. Coexpression of hKLF11 alone lack hKLF11. Similar to Fig. 1A, overexpression of hKLF11 alone had no impact on promoter activity. Coexpression of hPDX1 alone stimulated hInsP about 3.7fold (dotted line) in all fragments down to -173. Activity of the -101 + 54hInsP fragment was low due to loss of most enhancing elements but was still inducible by hPDX1 to a lesser extent. Coexpression of hKLFL1 and hPDX1 together synergistically and significantly stimulated the full length promoter as compared to PDX1 alone. This synergistic stimulation declines continuously with shortening of the promoter, and remains still significant after depletion of A5 (-254 + 54hInsP), but was abolished after removal of A3 (-173 + 54hInsP).

Because this observation suggests a relevant role for the PDX1 binding site A3, we further tested the impact of internally mutated PDX1 binding sites A3, GG2 and A1 within the context of the full length -881 + 54hInsP fragment (Fig. 3B). A5 was not included for absence of effects in 5'-deletion experiments (Fig. 3A). HEK293 cells were transiently cotransfected with wild type (wt) or mutated (mut) -881 + 54hInsP reporter plasmid, hp300, hPDX1 and hKLFL1 (0.25 μg each). Mock controls lack hKLFL1. Data were normalised to individual basal mock without hp300 and hPDX1 (dashed line), hKLFL1-mediated stimulation of hPDX1-induced wild type insulin promoter activity remains preserved after mutation of GG2 or A1 but was completely abolished by mutation of A3.

The CACCC box has been described to be essential for KLFL1/p300-mediated stimulation of hInsP activity in INS-1 β-cells (Bonnefond et al., 2011).

We tested the relevance of the CACCC box for hKLFL1-mediated stimulation of hInsP activity in HEK293 non-β-cells by site muta-
tion in the context of a -387 + 54hInsP fragment. For comparison, both wt and mutated sequences were cotransfected with hPDX1 alone or in combination with hKLFL1 (Fig. 3C). Data were normalised to basal wt mock without hPDX1 (dashed line). Reduced amount of transfected DNA resulted in reduced response compared to Fig. 3A (0.25 μg instead of 0.5 μg/plasmid). However, synergistic stimulation by PDX1 together with KLFL1 remains profound in wt hInsP but was completely absent upon CACCC box mutation. These data demonstrate that synergistic stimulation of hKLFL1/hPDX1-mediated hInsP activation in HEK293 non-β-cells requires the CACCC box and the PDX1 binding site A3, while deletion or mutation of the other PDX1 binding sites (A5, GG2 and A1) did not affect the stimulatory impact of KLFL1.

4. Discussion

KLFL1 has been characterised in different model systems as an activator as well as an inhibitor of the hInsP in β-cells. This indirectly suggests that the specific molecular context may contribute to these variations. Accordingly, the results of the present study demonstrate that hKLFL1 inhibits hInsP in β-cells but activates hInsP in HEK293 non-β-cells, which lack the β-cell-specific transcription machinery. hInsP activation in HEK293 non-β-cells requires ectopic expression of the otherwise absent major transcriptional insulin gene activator PDX1. The additionally required transcriptional cofactor p300 is present in HEK293. It has been demonstrated that several KLFs including KLFL1 (Zhang and Bieker, 1998; Zhang et al., 2001), KLFL2 (SenBanerjee et al., 2004), KLFL4 (Feinberg et al., 2005; Geiman et al., 2000) and KLFL13 (Song et al., 2003; Song et al., 2002) interact with p300 or its paralogue CBP. Based on these findings, we speculated in our previous report that also KLFL1 may interact with p300 in pancreatic β-cells. Meanwhile, zinc finger-binding of KLFL1 to p300 has been demonstrated (Fernandez-Zapico et al., 2009). Our present work confirms interactions of hKLFL1 with hp300 and further excludes interactions with hPDX1.

p300 levels have been described to be rate limiting for PDX1 function (Stanojevic et al., 2004). Consequently, we observed in HEK293 cells that hPDX1- and hPDX1/hKLFL1-induced hInsP activ-
ition were substantially enhanced if endogenous p300 levels were reduced in the absence of cotransfected hPDX1. We additionally coexpressed the established adenoviral p300 inhibitor E1A, which binds to p300 and thereby disrupts the formation of the p300:PDX1 transactivation complex (Qiu et al., 2002). Complete suppression of induced hInsP activation by E1A validates that KLF11-mediated inhibition of hInsP in INS-1E cells was achieved. We additionally cotransfected p300 and hKLF11 in INS-1E cells. The hInsP activity was substantially enhanced if endogenous p300 levels were reduced in the absence of cotransfected hPDX1. We additionally coexpressed p300 in INS-1E cells with hKLF11. This data suggest that interactions between KLF11 and p300 suppress PDX1-mediated transactivation of hInsP in β-cells. The regulatory elements A5, A3, GC2 and A1 are known PDX1 binding sites within the hInsP. Among these sites only the A3 element is required for KLF11 functions in HEK293 non-β-cells since its mutation abolished the observed synergistic stimulatory effect of pHD1X together with KLF11. The hInsP also contains two putative binding sites for KLF11; a GC-rich site at −348 to −340 and a CACCC motif at −96 to −88. In previous work, we detected binding of KLF11 to the GC-rich site (Niu et al., 2007) thereby confirming findings of Neve et al. (2005). Though initially expected, 5′-deletion of the GC site did not influence the inhibitory impact of KLF11 on hInsP activity in β-cells. Conversely, we found a reduced basal hInsP activity and also a reduced inhibitory impact of KLF11 when the CACCC box was mutated. Our current data reveal that a functional CACCC box is also required for the stimulatory impact of KLF11 on the hInsP in HEK293 non-β-cells. Since own earlier published gel shift analysis did not detect any binding of KLF11 to the CACCC box, we assume that KLF11 may interact indirectly via cofactors with this site.

Bonnefon et al. (2011) confirmed general loss of hInsP activity after CACCC box mutation, but in contrast demonstrated interactions between KLF11 and this site. They assumed that promoter activity depends on proper binding of KLF11 to the CACCC site while disruption of this binding causes reduced insulin gene expression. In support they demonstrated reduced insulin levels in otherwise healthy, fertile and unobtrusive KLF11−/− mice (Song et al., 2005). However, the global nature of the gene disruption makes it difficult to ascertain whether the reduced insulin gene expression in β-cells is caused by loss of KLF11 binding to the CACCC box or by yet unknown indirect mechanisms. Interestingly, the same study demonstrates decreased KLF11 activation of hInsP in β-cells if the inhibitor DN-p300 is ectopically produced (Bonnefon et al., 2011). This supports the current consensus that KLF11 interacts with p300.

hInsP-related hKLF11 functions require the p300:PDX1 transactivation complex as well as the PDX1 binding site A3 and also the CACCC box. Functional inactivation of one of these factors is sufficient to abolish the impact of KLF11. On this account, KLF11 appears to be a modulator of p300:PDX1-mediated hInsP transactivation. In HEK293 non-β-cells, KLF11 may support the incomplete p300:PDX1 transactivation complex in the absence of β-cell-specific cofactors and thereby enhances promoter activity. In β-cells, all specific cofactors are present and interactions between hKLF11 and p300 may impair complex formation and subsequently reduce PDX1-mediated transactivation.

In summary, our data demonstrate that KLF11 can differentially regulate the insulin gene depending on the molecular context. We could consistently uncover that KLF11 regulates hInsP via p300 and PDX1 independent of the molecular environment or type of action (stimulation in non-β-cells vs. inhibition in β-cells). This capability may, at least in part, explain opposite regulation of hInsP found by others in different experimental settings/models. Importantly, KLF11 functions require two regulatory sites within the hInsP; the A3 element and the CACCC box. In conclusion, we postulate that KLF11 and potentially other KLFs with redundant functions have a role in fine-tuning insulin gene expression in certain cellular situations rather than playing a major role as basic transcriptional activators or suppressors of this gene in vivo.

Acknowledgements

This work is dedicated to our colleague Xinje Niu who initiated the KLF11 project and passed away in December 2006 in China under tragic circumstances.

References


McConnell, B.B., Yang, V.W., 2010. Mammalian Kruppel-like factors in health and

Neve, B., Fernandez-Zapico, M.E., Ashkenazi-Katalan, V., Dina, C., Hamid, Y.H., Joly,
E., Vaillant, E., Benmerrou, V., Durand, E., Bakaher, N., Delannoy, N., Vaxillaire,
M., Cook, T., Dalingha-Thie, G.M., Jansen, H., Charles, M.A., Clement, K., Galan, P.,
Herberg, S., Helbecque, N., Charpentier, G., Prentki, M., Hansen, T., Pedersen, O.,
Urrutia, R., Melloul, D., Fraguell, P., 2005. Role of transcription factor KLF11 and
Acad. Sci. USA 102, 4807–4812.

Niu, X., Perakakis, N., Laubner, K., Limbert, C., Stahl, T., Brendel, M.D., Bretzel, R.G.,
proinsulin promoter activity in pancreatic beta cells. Diabetologia 50, 1433–
1441.

Ou, X.M., Chen, K., Shih, J.C., 2004. Dual functions of transcription factors,
transforming growth factor-beta-inducible early gene (TIEG)2 and Sp3, are
mediated by CACCC element and Sp1 sites of human monoamine oxidase

Qiu, Y., Guo, M., Huang, S., Stein, R., 2002. Insulin gene transcription is mediated by
interactions between the p300 coactivator and PDX-1, BET2, and E47. Mol. Cell

Schreiber, E., Matthias, P., Muller, M.M., Schaffner, W., 1989. Rapid detection of
octamer binding proteins with ‘mini-extracts’, prepared from a small number of
cells. Nucleic Acids Res. 17, 6419.

Subramaniam, M., Harris, S.A., Oursler, M.J., Rasmussen, K., Riggs, B.L., Spelsberg,
putative zinc finger protein in human osteoblasts. Nucleic Acids Res. 23,
4907–4912.

Tanahashi, T., Shinohara, K., Keshavarz, P., Yamaguchi, Y., Miyawaki, K., Kunika, K.,
Moritani, M., Nakamura, N., Yoshikawa, T., Shiota, H., Inoue, H., Itakura, M.,
2008. The association of genetic variants in Kruppel-like factor 11 and Type 2

Wollheim, C.B., 2001. Pdx1 level defines pancreatic gene expression pattern and

Zhang, W., Kadam, S., Emerson, B.M., Bieker, J.J., 2001. Site-specific acetylation by
p300 or CREB binding protein regulates erythroid Kruppel-like factor
(EKLF) activity by interaction with histone acetyltransferases. Proc. Natl.
Acad. Sci. USA 98, 9855–9860.

Zhang, W., Kadami, S., Emerson, B.M., Bieker, J.J., 2001. Site-specific acetylation by
p300 or CREB binding protein regulates erythroid Kruppel-like factor
transcriptional activity via its interaction with the SWI-SNF complex. Mol.
Cell Biol. 21, 2413–2422.

Please cite this article in press as: Perakakis, N., et al. Human Krüppel-like factor 11 differentially regulates human insulin promoter activity in β-cells and
non-β-cells via p300 and PDX1 through the regulatory sites A3 and CACCC box. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/
j.mce.2012.07.003