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WTIP interacts with ASXL2 and blocks ASXL2-mediated activation of retinoic acid signaling

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ABSTRACT

The Asx-like (ASXL) family proteins are chromatin factors that play dual roles in transcriptional activation and repression. ASXL2 is highly expressed in the heart and is required for proper heart development and function. Here, we identify a novel ASXL2-binding partner, the LIM domain-containing protein WTIP. Genetic and biochemical assays show a direct interaction between ASXL2 and WTIP. In HeLa cells, ASXL2 enhances retinoic acid-dependent luciferase activity, while WTIP represses it. Furthermore, WTIP blocks ASXL2's stimulatory effect on transcription. In addition, we found that ASXL2 and WTIP are expressed in mouse embryonic epicardial cells, a tissue that is regulated by retinoic acid signaling. Together, these results implicate ASXL2 and WTIP in regulation of retinoic acid signaling during heart development.

1. Introduction

The *Drosophila* Asx protein is a chromatin factor that plays dual roles in transcriptional activation and repression [1]. Asx has three mammalian homologs, Asx-like 1, 2 and 3 (ASXL1, ASXL2, and ASXL3). Mutations in ASXL family proteins have been implicated in a wide range of myeloid malignancies [2–4] and in Bohring–Opitz syndrome [5,6]. Using a mutant mouse model, we have previously shown that ASXL2 is highly expressed in the heart [7] and is involved in maintaining proper cardiac function [8]. More recently, we demonstrated a requirement for both ASXL1 and ASXL2 during normal heart development [9].

A number of studies have shown that Asx and ASXL proteins regulate chromatin configuration through functional interactions with two histone-modifying complexes, Polycomb repressive deubiquitinase (PR-DUB) [10] and Polycomb repressive complex 2 (PRC2) [11,12]. PR-DUB removes ubiquitin from mono-ubiquitinated histone H2A lysine 119 (uH2A). The deubiquitinase activity of PR-DUB was shown to be required for proper *Hox* gene repression in *Drosophila* [10]. PRC2 contains histone methyl-transferase activity and methylates lysine 27 of histone H3 (H3K27) [13]. Trimethylated H3K27 (H3K27me3) is a well-known mark of gene silencing.

The full-length ASXL2 protein contains several conserved domains separated by sequence-divergent regions. These include the HARE helix-turn-helix (HARE-HTH) predicted DNA-binding domain (aa10-88), the Asx-homology (ASXH) domain (aa215-344), a nuclear receptor (NR) binding motif (aa896-901), and the plant homeodomain (PHD) (aa1335-1366) [14–16]. In *Drosophila* Asx and mouse ASXL1, an N-terminal fragment containing HARE-HTH and ASXH was shown to mediate interaction with the histone deubiquitinase Calypso/BAP1 [10]. Consistent with this finding, our lab has shown that ASXL2 and BAP1 interact *in vivo* to regulate deubiquitination of uH2A [11]. We and others have also shown that ASXL1/2 interact with PRC2 and regulate PRC2 recruitment to target loci [11,12]. However, it is unclear which region of ASXL mediates this interaction.

There is also evidence that ASXL proteins interact with nuclear receptors. For example, ASXL1 has been shown to interact with the retinoic acid receptor RARα via an NR binding box and act as either co-activator or co-repressor of RA signaling in a cell type-dependent manner [16,17]. RA signaling plays important roles during heart development by regulating chamber formation and cardiomyocyte proliferation and maturation [18–20]. Since our lab has previously shown that ASXL2 is predominantly expressed in the heart [7] and required for heart morphogenesis [9], we hypothesize that ASXL2 may regulate RA signaling.

Here we present a study aimed at a better understanding of the functional mechanism of ASXL2. First, we screened for proteins that interact with ASXL2 outside the N-terminal region. We discovered

Abbreviations: Asx, additional sex comb; ASXH, Asx-homology; ASXL, additional sex comb-like; ATRA, all *trans*-retinoic acid; HARE-HTH, HARE helix-turn-helix; H3K27me3, trimethylated histone H3 lysine 27; NR, nuclear receptor; PHD, plant homeodomain; PRC2, Polycomb repressive complex 2; PR-DUB, Polycomb repressive deubiquitinase; RA, retinoic acid; RAR, retinoic acid receptor; uH2A, mono-ubiquitinated histone H2A; WTIP, Wilms tumor 1-interacting protein.

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genetic and biochemical evidence for a direct interaction between ASXL2 and Wilms Tumor 1-Interacting Protein (WTIP), a member of the ZYXIN family of LIM domain-containing proteins [21]. Secondly, we investigated the effect of ASXL2, with or without WTIP, on RA signaling.

2. Materials and methods

2.1. Plasmids

For yeast two-hybrid experiments, the following ASXL2 cDNAs were cloned in frame into pGBKT7 vector (Clontech), and expressed as GAL4-DBD fusion proteins: full-length ASXL2, N-terminal region ($ASXL2_{1-599}$), C-terminal region ($ASXL2_{600-1370}$), C-terminal region missing the PHD domain ($ASXL2_{600-1295}$), and PHD region alone ($ASXL2_{1313-1370}$). The prey plasmid isolated from the initial screen, pACT2-$hWTIP_{245-430}$, was used in all confirmation matings.

For co-immunoprecipitations (co-IPs) and luciferase assays, FLAG-$ASXL2_{720-1370}$ was inserted into pcDNA3 vector (Invitrogen). Myc-WTIP, Myc-NΔWTIP, and Myc-NΔZYXIN [22] were generous gifts from Dr. John R. Sedor (Case Western Reserve University). The WTIPΔLD2,3-HA construct [23] was a gift from Dr. Sigmar Stricker (Berlin, Germany). The LD2,3-HA construct was generated by inserting WTIP cDNA into pCS2 destination vector (gift from Dr. Hua Jin, University of Illinois at Chicago). The following reporter constructs were used in luciferase assays: RARE-tk-luciferase (gift from Dr. Rene Bernards, Netherlands Cancer Institute) and pSV-β-gal (Promega).

For GST-pulldown assays, GST-NΔWTIP [22] was a gift from Dr. Sedor (Case Western Reserve University). GST-PHD was constructed by inserting the PHD region of ASXL2 (aa1332-1370) into pGEX-6P-1 vector (GE Healthcare). For radiolabeling, $ASXL2_{600-1370}$, $ASXL2_{600-1295}$, and pcDNA3-WTIP [23] (gift from Dr. Stricker, Berlin, Germany) were used.

2.2. Yeast two-hybrid assays

The initial yeast two-hybrid screen was conducted using the Matchmaker System (Clontech). Bait plasmid pGBKT7-$ASXL2_{600-1370}$ was used to screen a pretransformed human heart cDNA library in pACT2 vector (Clontech). Mating and colony selection was performed according to the user's manual (Clontech). For mapping of interacting domains, bait and prey constructs were individually transformed into AH109 and Y187 yeast strains, respectively, and mated.

2.3. Co-immunoprecipitation

For co-IP experiments, mammalian HEK293 cells cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (HyClone) were transiently transfected with equal amounts of DNA using branched polyethylenimine (Sigma–Aldrich). Cells were harvested 48 h later and lysed in lysis buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 3% Triton-X 100) supplemented with protease inhibitor cocktail (Calbiochem). After centrifugation, the supernatants were used as input for IP and incubated with anti-FLAG M2 magnetic beads (Sigma) or anti-Myc agarose beads (Clontech). Immunoprecipitates were washed extensively in wash buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl) and bound proteins were eluted. Whole cell lysate, input, and IP fractions were used for SDS–PAGE followed by Western blot analysis.

2.4. GST-pulldown assay

GST and GST-fusion proteins were expressed in *Escherichia coli*. Radiolabeled proteins were obtained *in vitro* using the TNT Coupled Reticulocyte Lysate System (Promega) in the presence of [^{35}S]-methionine (Perkin–Elmer). [^{35}S]-labeled proteins were incubated with GST or GST-fusion proteins immobilized on glutathione-Sepharose 4 Fast Flow beads (GE Healthcare) supplemented with protease inhibitor cocktail (Calbiochem) and 100 μM ZnCl (Sigma). After incubation, the beads were washed extensively and bound proteins were separated via SDS–PAGE and visualized by autoradiography.

2.5. Luciferase reporter assays

HeLa cells were seeded in 24-well plates and transfected with RARE-tk-luc, pSV-β-gal, and constructs for ASXL2, WTIP or both using branched polyethylenimine (Sigma–Aldrich). Total amount of DNA was kept constant using pcDNA3 empty vector. After 48 h, cells were fed with DMEM containing 5% charcoal-stripped FBS (Life Technologies) and incubated overnight with or without 0.2 μM ATRA (Sigma). Cells were lysed using 1× passive lysis buffer (Promega) and luciferase activity was measured according to the Luciferase Reporter Assay (Promega). β-Galactosidase activity was assayed to normalize for transfection efficiency.

2.6. Immunostaining

Primary embryonic epicardial cells were isolated from E11.5 mouse hearts and cultured in DMEM supplemented with 10% FBS and primocin as described previously [24]. After 48 h, cells were fixed in methanol and stained with anti-WTIP polyclonal antibody (sc-241738; Santa Cruz Biotechnology) and visualized with Texas-Red-conjugated secondary antibody. Cells were double stained with Hoescht 33342 nuclear stain (Life Technologies).

E18.5 mouse heart frozen sections were fixed in methanol and stained with anti-WTIP antibody as mentioned above.

2.7. Reverse transcription (RT)-PCR

Total RNA was extracted from immortalized mouse embryonic epicardial cells [24] using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The cDNA was synthesized and amplified from 200 ng of total RNA using OneStep RT-PCR Kit (Qiagen) with primers specific to *Asxl2*: forward, 5′-CGAGCACTGATCAACAAGCAC-3′, reverse, 5′-TCTTGTCGAATTCT-CACCTGC-3′ or *Actin* control: forward, 5′-TCACCCACACTGTGCC-CATCT-3′, reverse, 5′-TGGTGAAGCTGTAGCCACGCT-3′.

3. Results

3.1. Genetic interactions between WTIP and ASXL2 in yeast

To identify protein partners that interact with ASXL2 outside of the N-terminus, a yeast two-hybrid screen was conducted using a cDNA corresponding to the C-terminal region ($ASXL2_{600-1370}$) (Fig. 1A). One of the positive clones isolated encoded a fragment of the human WTIP (Fig. 1B). WTIP contains an N-terminal proline-rich region with putative SH3 binding sites and a C-terminal region with three LIM domains [22]. The $ASXL2_{600-1370}$-interacting clone, hereafter referred to as $hWTIP_{245-430}$, encoded partial LIM 1 but complete LIM 2 and 3 of WTIP.

Confirmation matings showed that $hWTIP_{245-430}$ strongly interacts with $ASXL2_{600-1370}$, but not $ASXL2_{1-599}$ or full-length protein (Fig. 1C and D). Furthermore, strong interaction was detected with

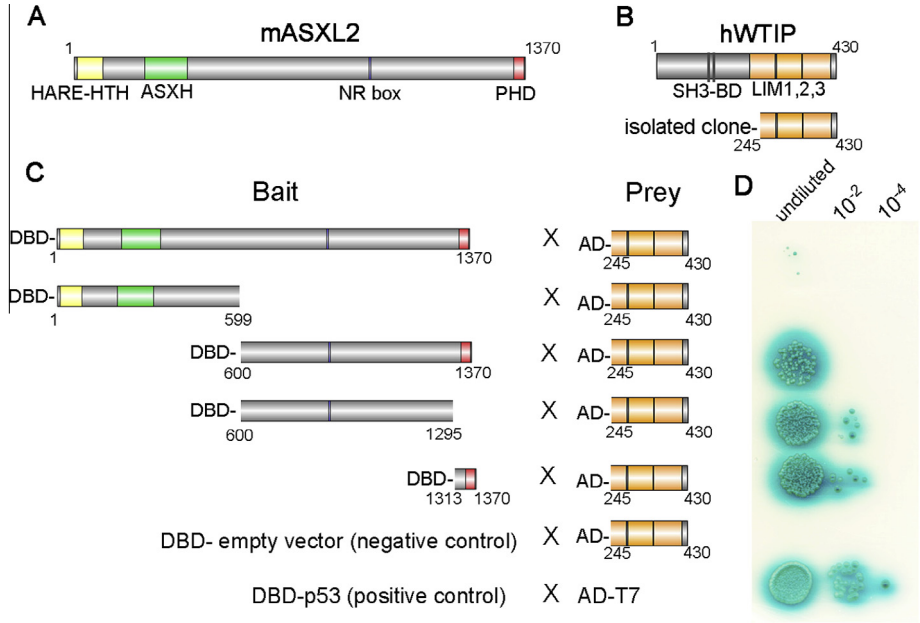

Fig. 1. ASXL2 interacts with WTIP in yeast. (A and B) Schematic representation of ASXL2 (A) and WTIP (B) proteins, showing their conserved domains. (C) Yeast two-hybrid matings using truncations of ASXL2 fused to Gal4-DNA binding domain (DBD) as bait and the isolated WTIP clone fused to GAL4-activation domain (AD) as prey. (D) Serial dilutions of the mating mixtures from (C) were plated on SD/-Ade/-His/-Leu/-Trp/X-α-Gal plates. The WTIP clone interacts with multiple parts on C-terminal ASXL2 but not N-terminal or full-length ASXL2. HARE-HTH: HARE-helix-turn-helix domain; ASXH, *Asx*-homology domain; NR, nuclear receptor box; PHD, plant homeodomain; SH3-BD, SH3 binding domain.

either the PHD finger alone ($ASXL2_{1313–1370}$), or a fragment missing the PHD finger ($ASXL2_{600–1295}$) (Fig. 1C and D), suggesting that the WTIP–ASXL2 interaction is mediated by at least two regions of ASXL2.

3.2. Biochemical characterization of ASXL2–WTIP interaction in mammalian cells

To determine whether ASXL2 and WTIP physically interact in mammalian cells, we performed reciprocal co-IP assays using FLAG-tagged ASXL2 and Myc-tagged mouse WTIP. Myc-WTIP co-IPed with FLAG-$ASXL2_{720–1370}$ in double transfected cells but not in single transfected control cells (Fig. 2A).

Next, we examined the specificity of the ASXL2–WTIP interaction. Since WTIP belongs to the ZYXIN family of proteins, members of which share high sequence similarity within the LIM regions [22], we decided to check whether ASXL2 interacted with the LIM domains of ZYXIN. While the LIM domains of WTIP (NΔWTIP) co-IPed with $ASXL2_{720–1370}$ and vice versa (Fig. 2B), the LIM domains of ZYXIN (NΔZYXIN) did not (Fig. 2C). This suggests that the ASXL2–WTIP interaction is specific to the LIM domains of WTIP.

Since ASXL2 interacts with LIM 2 and 3 of WTIP in yeast two-hybrid assays, we used co-IP assays to biochemically determine the role of the two LIM domains in the interaction. A WTIP fragment containing only LIM 2 and 3 (LD2,3-HA) co-IPed with FLAG-$ASXL2_{720–1370}$ (Fig. 2D), while a truncation missing LIM 2 and 3 (WTIPΔLD2,3-HA) did not (Fig. 2E), confirming that these domains are sufficient and necessary for interaction.

3.3. ASXL2 and WTIP interact directly in vitro

To determine whether ASXL2 and WTIP interact directly, GST-pulldown assays were performed. GST-NΔWTIP immobilized on glutathione-Sepharose beads retained *in vitro*-translated $ASXL2_{600–1370}$ and $ASXL2_{600–1295}$ (Fig. 2F). Furthermore, GST-PHD retained *in vitro*-translated WTIP (Fig. 2G). These results are consistent with results of the yeast two-hybrid assays (Fig. 1C and D). Together, they indicate that the PHD domain of ASXL2 and at least one other region outside the PHD domain directly interact with the LIM region of WTIP.

3.4. C-terminal ASXL2 enhances ligand-dependent retinoic acid signaling

The effect of ASXL2 on RA signaling was examined using a retinoic acid-response element (RARE)-driven luciferase reporter. $ASXL2_{720–1370}$ enhanced all-*trans* retinoic acid (ATRA)-induced luciferase activity in HeLa cells in a dose-dependent manner (Fig. 3A). Interestingly, full-length ASXL2 had no effect on RA signaling (Fig. 3B).

3.5. LIM domains 2 and 3 of WTIP block ASXL2's effect on retinoic acid signaling

WTIP has been shown to interact with RARα in HEK293 cells and repress RARE-dependent luciferase activity in P19 cells [25]. This prompted us to test the effect of WTIP on the increased RA signaling activity observed with $ASXL2_{720–1370}$ (Fig. 3C). HeLa cells were transfected with constructs for ASXL2, WTIP, or both. Similar to previous results, $ASXL2_{720–1370}$ alone enhanced ligand-dependent transcriptional activity. Conversely, WTIP alone repressed ATRA-induced transcription. When both ASXL2 and WTIP are present, WTIP blocks ASXL2's effect on luciferase transcription, suggesting that WTIP acts downstream of ASXL2 in the regulation of RA signaling.

We next tested which part of WTIP is important for repression of RA signaling. As shown in Fig. 3D and E, LD2,3-HA repressed ATRA-induced transcription while WTIPΔLD2,3-HA did not. These results suggest that LIM 2 and 3 of WTIP are sufficient and necessary for repressing RA signaling activity.

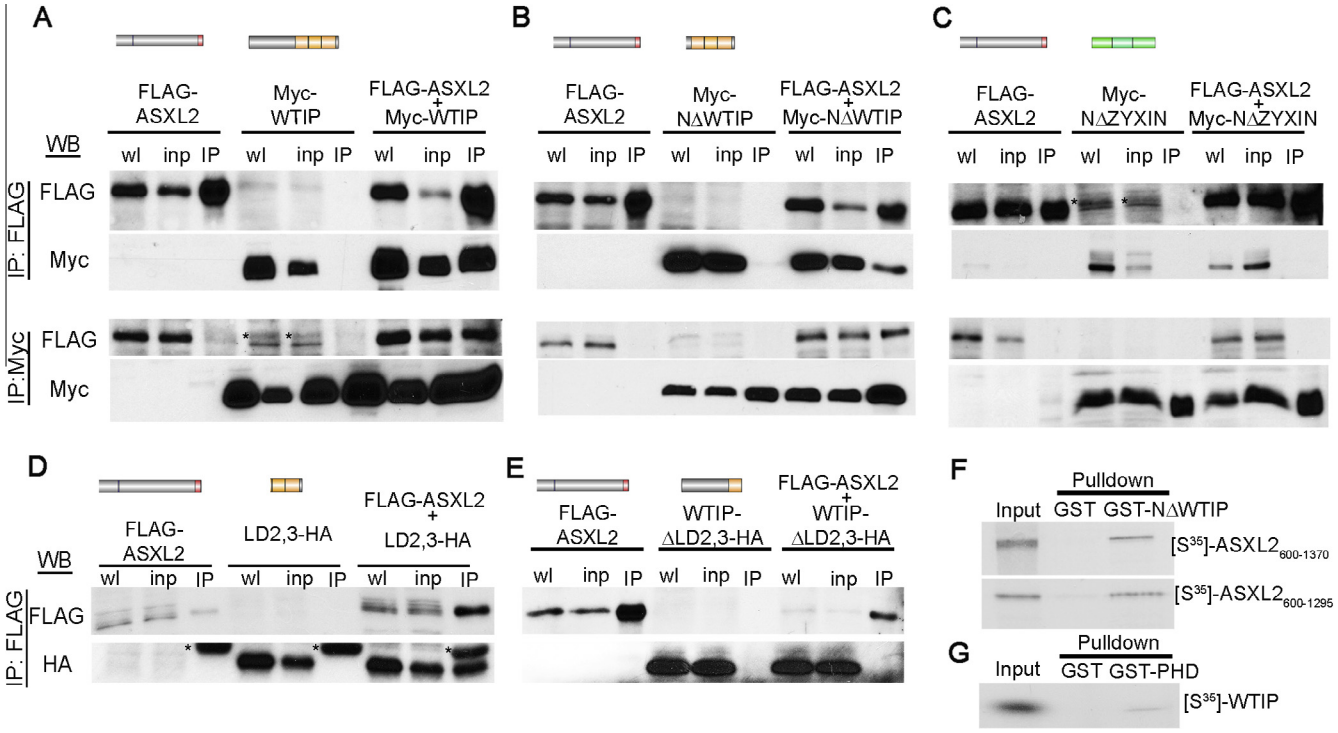

Fig. 2. Biochemical characterization of ASXL2 and WTIP interaction. (A–C) Reciprocal co-IP assays. Cells were transiently transfected with FLAG-tagged $ASXL2_{720-1370}$ and Myc-WTIP (A), Myc-NΔWTIP (B) or Myc-NΔZYXIN (C) constructs. Proteins were IPed using anti-FLAG or anti-Myc beads, as indicated. Whole lysate (wl), input (inp) and IP fractions were used for SDS–PAGE followed by Western blot (WB) analysis. (D and E) Co-IPs were conducted using FLAG-tagged $ASXL2_{720-1370}$ and LD2,3-HA (D) or WTIPΔLD2,3-HA (E). Proteins were IPed using anti-FLAG beads and analyzed by WB analysis. (F and G) GST-pulldown assays to examine direct interaction between GST-NΔWTIP and [S^{35}]-labeled ASXL2 constructs (F) and GST-PHD and [S^{35}]-labeled WTIP (G) (* Non-specific bands).

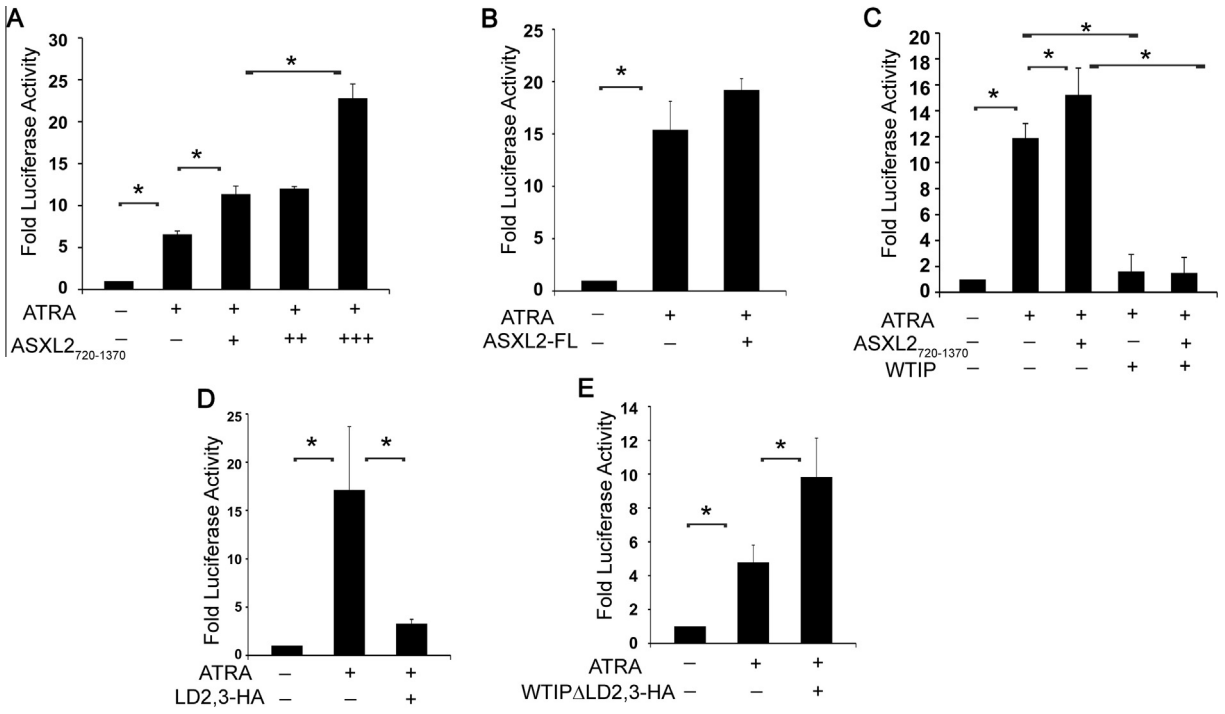

Fig. 3. ASXL2 and WTIP regulate RA-dependent luciferase activity. (A and B) HeLa cells were transfected with RARE-tk-luciferase and pSV-β-gal reporter constructs and $ASXL2_{720-1370}$ (A) or ASXL2-FL (B) with or without 0.2 μM ATRA. (C–E) HeLa cells were transfected with reporters, $ASXL2_{720-1370}$ and/or WTIP (C), LD2,3-HA (D) or WTIPΔLD2,3-HA (E) constructs with or without 0.2 μM ATRA. Luciferase activity was measured and normalized to β-gal activity. Error bars represent standard deviations from at least 3 independent experiments. ($^*p < 0.05$).

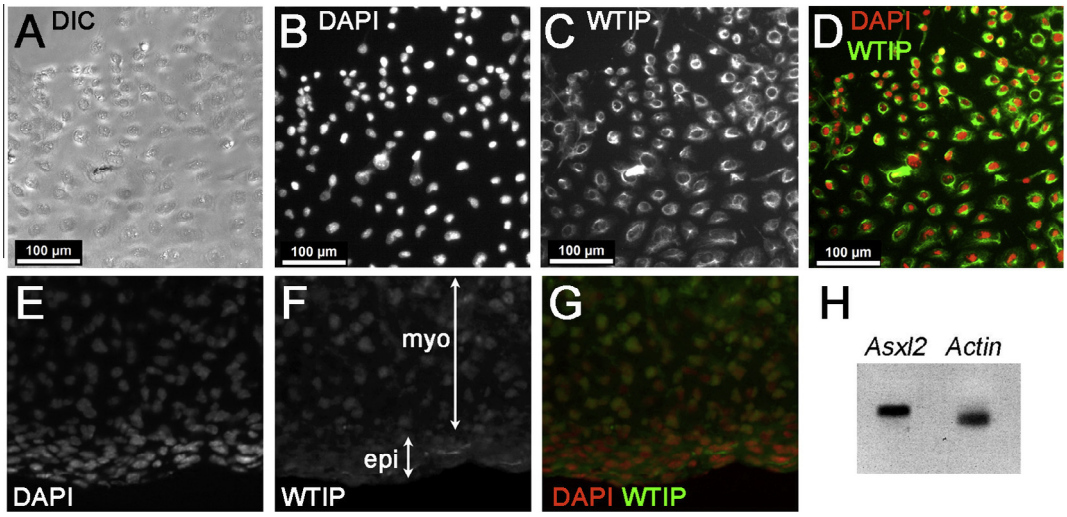

Fig. 4. WTIP and ASXL2 are expressed in embryonic epicardial cells. Primary epicardial cells cultured from E11.5 mouse hearts (A–D) and E18.5 mouse heart sections (E–G) were stained with WTIP antibody. Shown are DIC (A), DAPI (B and E), WTIP (C and F), and merged (D and G) images. (H) RT-PCR analysis of *Asxl2* and *Actin* control in mouse embryonic epicardial cell line. epi, epicardium; myo, myocardium.

3.6. Mouse embryonic epicardial cells express ASXL2 and WTIP

WTIP is known to interact with WT1, an important transcription factor that regulates epithelial-to-mesenchymal transition (EMT) in multiple tissues [26]. In the developing heart, WT1 is expressed specifically in the epicardium and regulates multiple signaling pathways, including RA signaling [27,28]. We asked whether WTIP is also expressed in the epicardium by immunostaining primary epicardial cells isolated from E11.5 mouse embryos. We detected robust WTIP expression in these cells (Fig. 4A–D). Interestingly, WTIP localizes to the cytoplasm and peri-nuclear regions but not in the nuclei.

Next we examined WTIP expression on frozen E18.5 heart sections (Fig. 4E–G). WTIP is expressed in both epicardium and myocardium. Cells in the myocardium exhibit strong nuclear localization of WTIP. In contrast, in the epicardium, WTIP is distributed in a diffused pattern without apparent nuclear localization.

We have previously shown that *Asxl2* is broadly expressed throughout the developing heart [7,9], however we have not specifically examined whether it is expressed in the epicardium. No existing ASXL2 antibodies detect endogenous levels of ASXL2 protein in immunostainings, therefore, we used RT-PCR to examine *Asxl2* expression in an immortalized epicardial cell line [24]. Robust *Asxl2* transcription was detected in these cells (Fig. 4H). Together, these results show that *Asxl2* and WTIP are both expressed in mouse embryonic epicardial cells, suggesting functional roles for these proteins in the epicardium.

4. Discussion

Mammalian ASXLs are large proteins ranging from 1370 to 2259 amino acids. Sequence analyses have identified several short conserved regions interspersed with long stretches of divergent sequences [14,29–31]. ASXL proteins do not have enzyme domains or sequence-specific DNA binding domains, suggesting that they likely function as co-factors for other proteins or as molecular scaffolds for multi-protein assemblies. Indeed, recent studies have identified several protein partners for ASXL family members, including the histone deubiquitinase BAP1 [10], the histone methyltransferase complex PRC2 [11], and the nuclear receptors RARα [16], PPARγ [32], and LXRα [33]. Here, we report the identification of another ASXL protein partner, WTIP. Using luciferase assays, we show that ASXL2 enhances, while WTIP inhibits, RA-induced transcription in HeLa cells. Furthermore, WTIP is able to block the stimulating effect of ASXL2 on RA signaling.

The ability of ASXL2 to enhance RA signaling in HeLa cells is consistent with previous reports that ASXL2 facilitates nuclear receptor-mediated signaling, namely PPARγ regulation in adipocytes [32] and LXRα regulation in hepatocytes [33]. In 3T3-L1 cells, ASXL2 has been shown to positively regulate signaling during adipogenesis by recruiting H3K9ac and H3K4me3, which are activating histone modifications, and the H3K4 methyltransferase MLL1 [32]. In HeLa cells, ASXL2 may be utilizing a similar mechanism to enhance RA signaling by recruiting activating histone marks to target gene promoters. Whether this is indeed the mechanism awaits future study.

Interestingly, while the C-terminus of ASXL2 is able to both interact with WTIP and activate RA signaling, the full-length ASXL2 does neither (Figs. 1D and 3B). One possibility is that the N-terminus of ASXL2 has an auto-inhibitory effect that interferes with the functions of the C-terminal region. This type of auto-regulatory mechanism has been well-characterized in several transcription factors and signaling proteins [34]. The inhibition may be relieved in the presence of a protein binding partner that induces conformational changes. Alternatively, full-length ASXL2 may require specific partners to fold properly and such partners may be lacking in yeast and HeLa cells. Consistent with this scenario, analysis of ASXL2 sequence using PONDR-FIT [35], a program that predicts intrinsically disordered proteins (IDPs), identified long stretches of disordered regions (data not shown). An important feature of IDPs is that the whole or part of the protein transitions to a more ordered structure upon binding the right partner [36].

The co-expression of ASXL2, WTIP, and WT1 in embryonic epicardial cells raises the possibility that functional interactions between these proteins may be part of the mechanism regulating RA signaling during heart development. Consistent with their roles in transcriptional regulation, both ASXL2 and WT1 are nuclear proteins [8,37]. On the other hand, WTIP has been shown to shuttle between the nuclei and cytoplasm [22,38]. Curiously, WTIP localizes to nuclei in the myocardium, but not in the epicardium or in primary epicardial cells (Fig. 4A–G). This difference in localization suggests a mechanism for differential regulation of RA signaling within the heart. We expect ASXL2 to enhance RA signaling in

the epicardium, where WTIP is cytoplasmic. On the other hand, the activating effect of ASXL2 would be blocked by nuclear WTIP in the myocardium. Finally, it is conceivable that the spatial pattern of WTIP localization is regulated by developmental signals, providing further means to dynamically regulate RA signaling in the heart.

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References

[1] T.A. Milne, D.A. Sinclair, H.W. Brock, The Additional sex combs gene of Drosophila is required for activation and repression of homeotic loci, and interacts specifically with Polycomb and super sex combs, Mol. Gen. Genet. 261 (1999) 753–761.
[2] N. Carbuccia, A. Murati, V. Trouplin, M. Brecqueville, J. Adélaïde, J. Rey, et al., Mutations of ASXL1 gene in myeloproliferative neoplasms, Leukemia 23 (2009) 2183–2186.
[3] J. Boultwood, J. Perry, A. Pellagatti, M. Fernandez-Mercado, C. Fernandez-Santamaria, M.J. Calasanz, et al., Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia, Leukemia 24 (2010) 1062–1065.
[4] W.-C. Chou, H.-H. Huang, H.-A. Hou, C.-Y. Chen, J.-L. Tang, M. Yao, et al., Distinct clinical and biological features of de novo acute myeloid leukemia with additional sex comb-like 1 (ASXL1) mutations, Blood 116 (2010) 4086–4094.
[5] A. Hoischen, B.W.M. van Bon, B. Rodríguez-Santiago, C. Gilissen, L.E.L.M. Vissers, P. de Vries, et al., De novo nonsense mutations in ASXL1 cause Bohring–Opitz syndrome, Nat. Genet. 43 (2011) 729–731.
[6] M.N. Bainbridge, H. Hu, D.M. Muzny, L. Musante, J.R. Lupski, B.H. Graham, et al., De novo truncating mutations in ASXL3 are associated with a novel clinical phenotype with similarities to Bohring–Opitz syndrome, Genome Med. 5 (2013) 11.
[7] H.A. Baskind, L. Na, Q. Ma, M.P. Patel, D.L. Geenen, Q.T. Wang, Functional conservation of Asxl2, a murine homolog for the Drosophila enhancer of trithorax and polycomb group gene Asx, PLoS One 4 (2009) e4750.
[8] H.-L. Lai, M. Grachoff, A.L. McGinley, F.F. Khan, C.M. Warren, S.A.K. Chowdhury, et al., Maintenance of adult cardiac function requires the chromatin factor Asxl2, J. Mol. Cell. Cardiol. 53 (2012) 734–741.
[9] A.L. McGinley, Y. Li, Z. Deliu, Q.T. Wang, Additional sex combs-like family genes are required for normal cardiovascular development, Genesis 52 (2014) 671–686.
[10] J.C. Scheuermann, A.G. de Ayala Alonso, K. Oktaba, N. Ly-Hartig, R.K. McGinty, S. Fraterman, et al., Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB, Nature 465 (2010) 243–247.
[11] H.-L. Lai, Q.T. Wang, Additional sex combs-like 2 is required for polycomb repressive complex 2 binding at select targets, PLoS One 8 (2013) e73983.
[12] O. Abdel-Wahab, M. Adli, L.M. LaFave, J. Gao, T. Hricik, A.H. Shih, et al., ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression, Cancer Cell 22 (2012) 180–193.
[13] B. Czermin, R. Melfi, D. McCabe, V. Seitz, A. Imhof, V. Pirrotta, Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites, Cell 111 (2002) 185–196.
[14] L. Aravind, L.M. Iyer, The HARE-HTH and associated domains: novel modules in the coordination of epigenetic DNA and protein modifications, Cell Cycle 11 (2012) 119–131.
[15] C. Fisher, A human homolog of Additional sex combs, ADDITIONAL SEX COMBS-LIKE 1, maps to chromosome 20q11, Gene 306 (2003) 115–126.
[16] Y.-S. Cho, E.-J. Kim, U.-H. Park, H.-S. Sin, S.-J. Um, Additional sex comb-like 1 (ASXL1), in cooperation with SRC-1, acts as a ligand-dependent coactivator for retinoic acid receptor, J. Biol. Chem. 281 (2006) 17588–17598.
[17] S.-W. Lee, Y.-S. Cho, J.-M. Na, U.-H. Park, M. Kang, E.-J. Kim, et al., ASXL1 represses retinoic acid receptor-mediated transcription through associating with HP1 and LSD1, J. Biol. Chem. 285 (2010) 18–29.
[18] P. Kastner, N. Messaddeq, M. Mark, O. Wendling, J.M. Grondona, S. Ward, et al., Vitamin A deficiency and mutations of RXRalpha, RXRbeta and RARalpha lead to early differentiation of embryonic ventricular cardiomyocytes, Development 124 (1997) 4749–4758.
[19] K. Niederreither, J. Vermot, N. Messaddeq, B. Schuhbaur, P. Chambon, P. Dollé, Embryonic retinoic acid synthesis is essential for heart morphogenesis in the mouse, Development 128 (2001) 1019–1031.
[20] I. Stuckmann, S. Evans, A.B. Lassar, Erythropoietin and retinoic acid, secreted from the epicardium, are required for cardiac myocyte proliferation, Dev. Biol. 255 (2003) 334–349.
[21] J.L. Kadrmas, M.C. Beckerle, The LIM domain: from the cytoskeleton to the nucleus, Nat. Rev. Mol. Cell Biol. 5 (2004) 920–931.
[22] M.B. Srichai, M. Konieczkowski, A. Padiyar, D.J. Konieczkowski, A. Mukherjee, P.S. Hayden, et al., A WT1 co-regulator controls podocyte phenotype by shuttling between adhesion structures and nucleus, J. Biol. Chem. 279 (2004) 14398–14408.
[23] N.V. van Wijk, F. Witte, A.C. Feike, A. Schambony, W. Birchmeier, S. Mundlos, et al., The LIM domain protein Wtip interacts with the receptor tyrosine kinase Ror2 and inhibits canonical Wnt signalling, Biochem. Biophys. Res. Commun. 390 (2009) 211–216.
[24] A.F. Austin, L.A. Compton, J.D. Love, C.B. Brown, J.V. Barnett, Primary and immortalized mouse epicardial cells undergo differentiation in response to TGFbeta, Dev. Dyn. 237 (2008) 366–376.
[25] Z. Hou, H. Peng, D.E. White, D.G. Negorev, G.G. Maul, Y. Feng, et al., LIM protein Ajuba functions as a nuclear receptor corepressor and negatively regulates retinoic acid signaling, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 2938–2943.
[26] P. Hohenstein, N.D. Hastie, The many facets of the Wilms' tumour gene, WT1, Hum. Mol. Genet. 15 (2006) R196–R201.
[27] B. Zeng, X. Ren, F. Cao, X. Zhou, J. Zhang, Developmental patterns and characteristics of epicardial cell markers Tbx18 and Wt1 in murine embryonic heart, J. Biomed. Sci. 18 (2011) 67.
[28] A. von Gise, B. Zhou, L.B. Honor, Q. Ma, A. Petryk, W.T. Pu, WT1 regulates epicardial epithelial to mesenchymal transition through β-catenin and retinoic acid signaling pathways, Dev. Biol. 356 (2011) 421–431.
[29] C.L. Fisher, F. Randazzo, R.K. Humphries, H.W. Brock, Characterization of Asxl1, a murine homolog of Additional sex combs, and analysis of the Asx-like gene family, Gene 369 (2006) 109–118.
[30] M. Katoh, M. Katoh, Identification and characterization of ASXL2 gene in silico, Int. J. Oncol. 23 (2003) 845–850.
[31] M. Katoh, M. Katoh, Identification and characterization of ASXL3 gene in silico, Int. J. Oncol. 24 (2004) 1617–1622.
[32] U.-H. Park, S.K. Yoon, T. Park, E.-J. Kim, S.-J. Um, Additional sex comb-like (ASXL) proteins 1 and 2 play opposite roles in adipogenesis via reciprocal regulation of peroxisome proliferator-activated receptor gamma, J. Biol. Chem. 286 (2011) 1354–1363.
[33] U.-H. Park, M. Seong, E.-J. Kim, W. Hur, S.W. Kim, S.K. Yoon, et al., Reciprocal regulation of LXRα activity by ASXL1 and ASXL2 in lipogenesis, Biochem. Biophys. Res. Commun. 443 (2014) 489–494.
[34] M.A. Pufall, B.J. Graves, Autoinhibitory domains: modular effectors of cellular regulation, Annu. Rev. Cell Dev. Biol. 18 (2002) 421–462.
[35] B. Xue, R.L. Dunbrack, R.W. Williams, A.K. Dunker, V.N. Uversky, PONDR-FIT: a meta-predictor of intrinsically disordered amino acids, Biochim. Biophys. Acta 1804 (2010) 996–1010.
[36] L.M. Espinoza-Fonseca, Reconciling binding mechanisms of intrinsically disordered proteins, Biochem. Biophys. Res. Commun. 382 (2009) 479–482.
[37] A.W. Moore, L. McInnes, J. Kreidberg, N.D. Hastie, A. Schedl, YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis, Development 126 (1999) 1845–1857.
[38] J.H. Kim, M. Konieczkowski, A. Mukherjee, S. Schechtman, S. Khan, J.R. Schelling, et al., Podocyte injury induces nuclear translocation of WTIP via microtubule-dependent transport, J. Biol. Chem. 285 (2010) 9995–10004.