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Cell Regeneration

Nuclear-cytoplasmic shuttling of class IIa histone deacetylases regulates somatic cell reprogramming

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ABSTRACT

Class IIa histone deacetylases (HDACs) are a subfamily of HDACs with important functions in development and adult tissue homeostasis. As opposed to other HDACs, they lack catalytic function and bind transcription factors to recruit transcriptional co-regulators, mostly co-repressors such as nuclear receptor co-repressor (NCoR)/silencing mediator of retinoid and thyroid hormone receptor (SMRT). Class IIa HDACs enhance mouse somatic cell reprogramming to induced pluripotent stem cells (iPSCs) by repressing the function of the pro-mesenchymal transcription factor myocyte enhancer factor 2 (MEF2), which is upregulated during this process. Here, we describe, using HDAC4 and 7 as examples, that class IIa HDACs exhibit nuclear-cytoplasmic trafficking in reprogramming, being mostly cytoplasmic in donor fibroblasts and intermediate cells but translocating to the nucleus in iPSCs. Importantly, over-expressing a mutant form of HDAC4 or 7 that becomes trapped in the nucleus enhances the early phase of reprogramming but is deleterious afterwards. The latter effect is mediated through binding to the exogenous reprogramming factors at pluripotency loci, and the subsequent recruitment of NCoR/SMRT co-repressors. Thus, our findings uncover a context-dependent function of class IIa HDACs in reprogramming and further reinforce the idea that recruitment of co-repressors by the exogenous factors is a major obstacle for reactivating the pluripotency network in this process.

1. Introduction

Over-expression of exogenous transcription factors (e.g., the four Yamanaka factors: OCT4, SOX2, KLF4, and c-MYC; OSKM) in somatic cells can convert them to an embryonic stem cell (ESC)-like state.[1] These cells are named iPSCs and have great potential for regenerative medicine, toxicology screening, and *in vitro* disease modeling.[2–4] In addition, somatic cell reprogramming provides a remarkable model to understand cell fate transitions in other contexts such as development, cancer, and aging.[5–8] Reprogramming requires comprehensive cellular rearrangements that have two major goals: a) loss of somatic cell characteristics, which in mesenchymal-like cells is associated with the acquisition of an epithelial phenotype,[9] and b) the reactivation of the pluripotency network.[10–12] To achieve these goals, reprogramming cells must

undergo profound epigenetic changes that progressively reshape the cellular transcriptional landscape.[5,13,14] Among these changes, the post-translational modification of histones (e.g., methylation, acetylation, and phosphorylation) is a critical regulator of chromatin accessibility, facilitating or prohibiting the binding of the exogenous and endogenous pluripotency transcription factors.[13,15–17]

Histone acetylation generally serves as a marker for open chromatin and active gene expression. Accordingly, ESCs/iPSCs display much higher levels of histone acetylation than somatic cells.[13,18] Histone acetylation is governed by the balance between histone acetyltransferases and HDACs.[19] Notably, several reports have demonstrated that inhibiting HDAC activity with pan-HDAC inhibitors (e.g., valproic acid and sodium butyrate) potently enhances reprogramming efficiency.[20,21] However, the role of the individual HDACs in reprogramming is complex and has not yet been fully elucidated. There are 11 members in the canonical mammalian HDAC family, which are classified into three classes (class I, II, and IV) based on phylogenetic analysis and sequence similarity. The sirtuin family (SIRT1-7) of atypical HDACs constitutes the class III subfamily.[22] Both class IIa (HDAC4, 5, 7, and 9) and class III are unaffected by pan-HDAC inhibitors.[23] Recently, we found that HDAC3, a member of class I HDACs (HDAC1, 2, 3, and 8) acts as a barrier for OSKM reprogramming through recruitment of NCoR/SMRT co-repressors.[24] Therefore, suppressing the function of HDAC3 or NCoR/SMRT significantly enhances OSKM reprogramming efficiency and kinetics. We have also reported that over-expressing HDAC4, 5, and 7 enhances three factor-reprogramming (OCT4, SOX2, and KLF4; OSK) efficiency, whereas their knockdown has the opposite effect.[25] Class IIa HDACs act by promoting the mesenchymal-to-epithelial transition (MET) in the early phase of reprogramming. They do so by binding to and repressing the function of the developmental transcription factor MEF2, which is potently induced in reprogramming and activates the expression of pro-mesenchymal genes such as *Tgfb1*, *2*, and *3*.[25] Compared to class I HDACs, class IIa HDACs lack catalytic function and act instead by bridging transcription factors and chromatin regulators including class I HDACs themselves.[26,27] Notably, class IIa HDACs also differ from class I HDACs in terms of subcellular localization, as they can shuttle between cytoplasm and nucleus, whereas class I HDACs are mainly present in the nucleus.[28,29]

In this study, we have further characterized the function of class IIa HDACs in reprogramming, demonstrating that dynamic changes in their subcellular distribution influence their activity in reprogramming.

2. Materials and methods

2.1. Cell culture and reprogramming experiments

PlatE cells, HEK293T cells, and OG2[30] mouse embryonic fibroblasts were maintained in DMEM-HIGH GLUCOSE supplemented with 10% fetal bovine serum (Biowest). OG2 mouse ESCs were cultured on feeders (fibroblasts treated with mitomycin C) in standard ESC serum medium (DMEM supplemented with 15% fetal bovine serum (Biological Industries), non-essential amino acids, GlutaMAX™, sodium-pyruvate, penicillin/streptomycin, 0.1 mM β-mercaptoethanol, and 1000 U/ml leukemia inhibitory factor (LIF, Millipore)). Reprogramming experiments were performed as previously described[31] in standard ESC serum medium or ESC serum medium supplemented with 50 μg/ml vitamin C (Vc) (Sigma), as indicated. Doxycycline (Sigma) was used at 1 μg/ml for the indicated times. GFP+ colonies were visualized and counted using a Zeiss SteREO Lumar V12 microscope. Alkaline phosphatase (AP) activity staining was done as previously described.[31]

2.2. Plasmids and virus transduction

Retroviral vectors (pMXs-based) separately expressing OSKM were purchased from Addgene. All other constructs were made by us using cDNA obtained from mouse fibroblasts or purchased from Addgene, and cloned into pMXs or pRlenti-based lentiviral vector. DNA mutagenesis or deletion was produced using suitable oligos and a PCR-based method. shRNA inserts were cloned into pRetroSuper vectors. shRNA target sequences are listed in Table S1. For reprogramming experiments, mouse embryonic fibroblasts were seeded onto 12-well plates and incubated with retrovirus-containing supernatants generated by PlatE cells and/or lentivirus containing supernatants generated by HEK293T cells. First, we transduced mouse embryonic fibroblasts once with OSKM retroviruses for 12 h, and then with the lentiviruses for another 3 h or with shRNA retroviruses for another 24 h.

2.3. Immunoprecipitation, Western blotting, and immunofluorescence

For immunoprecipitation, cells were lysed on ice in TNE buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% Tergitol-type NP-40, 1 mM EDTA, and protease inhibitors). Anti-FLAG M2 magnetic beads (M8823, Sigma) were incubated overnight with cell lysate fractions. Samples were then washed 6 times with TBS. Western blotting was performed following standard principles and immunofluorescence was assessed using a Leica TCS SP2 spectral confocal microscope. Nuclear-cytoplasmic fractionation was performed following standard protocol. The following primary antibodies were used: anti-FLAG (F7425, Sigma), anti-NCoR (ABE251, Millipore), anti-HA (H6908, Sigma), anti-SMRT (ab24551, Abcam), anti-HDAC7 (ab12174, Abcam), anti-E-cadherin (BD Biosciences), anti-SSEA-1 (MC480, Cell Signaling), anti-SOX2 (MAB2018, R&D Systems), anti-OCT4 (sc-8628, Santa Cruz), anti-KLF4 (AF3158, R&D Systems), anti-MYC (AF3696, R&D Systems), anti-histone H3 (ab1791, Abcam), anti-ACTIN (A5316, Sigma), and anti-GAPDH (G8795, Sigma). DAPI was purchased from Sigma (D9542).

2.4. RNA isolation and quantitative real-time PCR (RT-qPCR)

Total RNA was isolated using TRIzol™ (Invitrogen). RT-qPCR analysis was performed using SYBR Green (Takara) and an ABI 7500 real-time PCR machine (Applied Biosystems). Samples were run in triplicates and normalized on the basis of *Gapdh* values. Primers are listed in Table S1.

2.5. Chromatin immunoprecipitation (ChIP) coupled with quantitative PCR (ChIP-qPCR)

ChIP was performed as following: cells were cross-linked in 1% formaldehyde for 10 min at room temperature and then quenched in 125 mM glycine for 5 min at room temperature. Samples were lysed in 1% SDS lysis buffer for 20 min at 4 °C, and then fragmented with a bioruptor (Diagenode) sonicator at 4 °C using high amplitude and 30 s ON and 30 s OFF cycles to produce size ranges between 200 and 500 base pairs. Two micrograms of each antibody were pre-bound by incubating with Protein A+G Dynabeads (Thermo Fisher Scientific) in PBST buffer for 6 h at 4 °C. Washed beads were added to the chromatin lysate and incubated overnight. Samples were washed twice with low salt washing buffer, twice with high salt washing buffer, twice with LiCl buffer, twice with TE supplemented with 50 mM NaCl, and eluted in elution buffer. Eluates were de-crosslinked at 65 °C for 6–15 h. Samples were then

treated with RNase A and proteinase K. DNA was purified with phenol:chloroform:isoamyl alcohol and used for RT-qPCR. ChIP-qPCR primers are listed in Table S1. The following antibodies were used for ChIP experiments: anti-IgG (A7016, Beyotime), anti-FLAG (F1804, Sigma), and anti-HA (H6908, Sigma).

2.6. MEF2-responsive reporter assay

HEK293T cells were transfected with a luciferase-based MEF2-responsive reporter (Panomics); a Renilla Luciferase reporter plasmid (Promega) was co-transfected as an internal control. Forty-eight hours after transduction, cells were harvested and analyzed for luciferase activity using a Veritas Luminometer machine.

3. Results

3.1. Nuclear class IIa HDACs block OSKM-mediated reprograming

Class IIa HDACs are a group of transcriptional regulators that shuttle between nucleus and cytoplasm, repressing gene expression only when translocated into the nucleus.[27] We initiated our work aiming to study whether the subcellular localization of HDAC7, a representative class IIa HDAC, changes in the context of reprogramming as it does for example during development.[29] We first investigated HDAC7 localization in mouse embryonic fibroblasts and ESCs using immunofluorescence and Western blotting. Interestingly, HDAC7 was mainly (though not exclusively) cytoplasmic in fibroblasts but nuclear in ESCs (Fig. 1A and B), indicating that there must be a translocation from the cytoplasm to the nucleus in the course of reprogramming. We then focused on OSKM reprogramming because the basal efficiency is higher than OSK reprogramming. We over-expressed wild-type HDAC7 (HDAC7wt) alongside OSKM in fibroblasts using pMXs-based retroviral vectors and assessed total HDAC7 localization in a serial time course experiment of reprogramming. Similar to endogenous HDAC7 in mouse embryonic fibroblasts, exogenous HDAC7wt was mainly cytoplasmic at the reprogramming time points measured, including the onset of activation of the transgenic Oct4-GFP reporter at day 10 (Fig. 1C and D). However, HDAC7 translocated into the nucleus in fully reprogrammed iPSCs (Fig. 1C), consistent with the pattern observed in ESCs (Fig. 1A). We thus hypothesized that forced expression of HDAC7 inside the nucleus during reprogramming would influence the outcome of this process. To test this, we generated a phosphorylation-defective HDAC7 mutant (HDAC7sa: serine 178, 344, and 479 substituted with alanine) that is incapable of nuclear export[32] and so is kept inside the nucleus throughout the reprogramming process (Fig. 1E). Strikingly, HDAC7sa did not affect the appearance of early colonies tested at day 7 (Fig. 1F), but almost completely prevented the formation of Oct4-GFP⁺ colonies at the end stage of reprogramming (Fig. 1F and G). The lack of a complete effect might be because these colonies originate from cells that were not transduced with the HDAC7sa vector or due to a compensatory mechanism. Conversely, HDAC7wt did not significantly influence early colony formation or Oct4-GFP⁺ colony formation compared to the control (Fig. 1F and G), in agreement with our previous report.[25] Similar results were observed when Vc was added to the serum-based medium to increase reprogramming efficiency (Fig. 1G),[31] or when we over-expressed the wild-type and a mutant form of HDAC4 (serine 245, 465, and 629 substituted with alanine) instead of the wild-type and mutant form of HDAC7 (Fig. 1H and I). We concluded that class IIa HDACs are actively, yet not completely, excluded from the nucleus during reprogramming and that sustained nuclear translocation derails the late phase of reprogramming.

3.2. Nuclear HDAC7 enhances the MET but represses pluripotency genes in reprogramming

To understand why constitutively nuclear class IIa HDACs block OSKM reprogramming, we employed a doxycycline-inducible lentiviral construct of HDAC7sa (iHDAC7sa) (Fig. 2A). Activation of iHDAC7sa in the first days of reprogramming (day 3-day 7) increased GFP⁺ colony formation ~3-fold on average without Vc and only moderately with Vc (Fig. 2B). Conversely, activation of iHDAC7sa in the late stage (day 8-day 17) reduced GFP⁺ colony formation (Fig. 2C). This pattern correlates with the observations above using constitutively nuclear HDAC7sa (see Fig. 1F and G). Notably, the loss of somatic cell characteristics and the MET are key events in the early phase of reprogramming,[9] whereas the reactivation of pluripotency genes characterizes the late phase.[10–12,14,33] Thus, we hypothesized that nuclear class IIa HDACs, using HDAC7 as an example, affect OSKM reprogramming outcome differently in the early stage versus late stage due to context-dependent effects in these phases. To study this, we monitored the effect of HDAC7sa on the transition from a somatic phenotype to the MET, and then the reactivation of the pluripotency gene network, using RT-qPCR. HDAC7sa and less significantly HDAC7wt reduced the expression of somatic genes (including Thy1) and enhanced epithelial genes (including Cdh1, encoding E-cadherin) at the early stage (Fig. 2D–F). This is consistent with the previously identified role of class IIa HDACs in promoting the MET in OSK reprogramming by repressing components of the TGF-β pathway.[25] In addition, HDAC7sa, and less noticeably HDAC7wt, enhanced cell proliferation (Figure S1A). Of note, proliferation is known to enhance reprogramming by increasing the chance of probabilistic events that facilitate the process.[34] This strongly suggests that HDAC7sa does not block reprogramming by affecting proliferation. HDAC7sa also blunted the acquisition of both early: Fut9 (whose gene product synthesizes SSEA-1) and AP activity (Fig. 2G and H), and late (e.g., endogenous Oct4 and Nanog) pluripotent cell markers (Fig. 2I) measured at day 8 and 10, whereas HDAC7wt displayed only a modest negative effect. Hence, nuclear class IIa HDACs have dual effects on reprogramming, beneficial or detrimental, depending on the phase, suggesting that they act through binding to different sets of transcription factors in each situation.

3.3. Nuclear HDAC7 interacts with OSKM and recruits NCoR/SMRT to repress pluripotency genes

We next investigated how nuclear HDAC7 prevents the reactivation of pluripotency genes in the late phase of reprogramming. Class IIa HDACs lack intrinsic DNA-binding capability and their interaction with transcription factors dictates target specificity.[35] We predicted that, in addition to MEF2 factors in the early phase of reprogramming, HDAC7 binds to OSKM and represses their activity to derail pluripotency gene reactivation. To explore this idea, we over-expressed OSKM factors individually in HEK293T cells together with HDAC7sa, detecting robust interaction by immunoblotting in all cases when either the exogenous factors or HDAC7sa were used as bait (Fig. 3A and S1B). Importantly, we confirmed this interaction in fibroblasts undergoing reprogramming with OSKM and HDAC7sa (Fig. 3B). Moreover, ChIP-qPCR demonstrated that exogenous OCT4 and HDAC7sa co-bind to multiple pluripotency gene loci including Oct4, Nanog, Esrrb, and Zfp42 (Rex1) (Fig. 3C and S1C). Hence, HDAC7sa binds to OSKM and is recruited to pluripotency loci in reprogramming, rather than acting by preventing the access of OSKM to their cognate sequences.

In multiple contexts, class IIa HDACs exert their repressive function by attracting co-repressors including NCoR and SMRT to target sites.[27] To assess whether the negative role of nuclear HDAC7
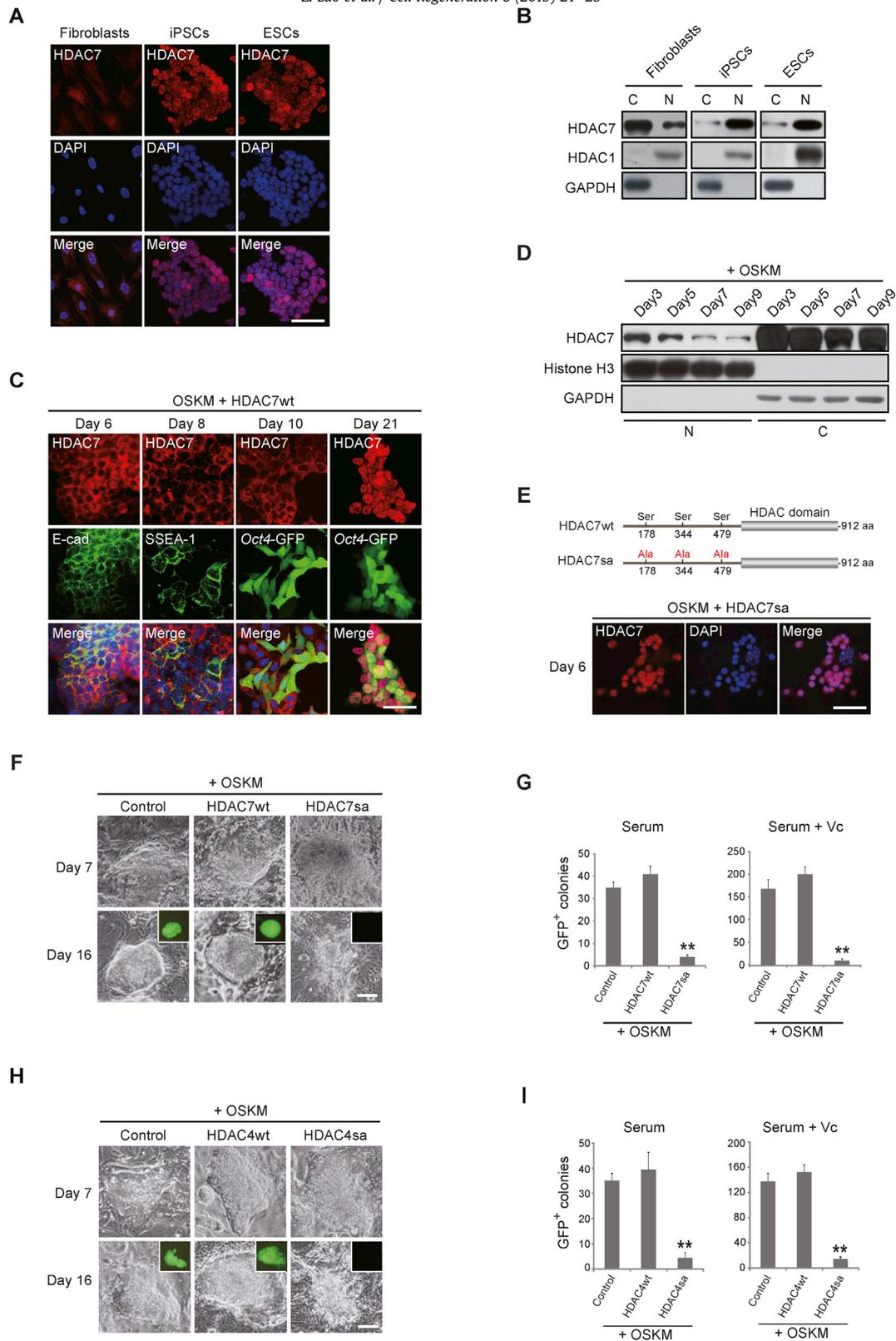
Fig. 1. Nuclear-cytoplasmic trafficking of class IIa HDACs in reprogramming. (A) Immunofluorescence for HDAC7 in fibroblasts, iPSCs, and ESCs. Nuclei stained with DAPI are in blue. Scale = 50 μm. (B) Immunoblot for HDAC7 in the cytoplasmic (C) fraction versus the nuclear (N) fraction of fibroblasts, iPSCs, and ESCs. HDAC1 and GAPDH are loading controls for the nuclear fraction and cytoplasmic fractions, respectively. (C) Double immunostaining for HDAC7 or the indicated proteins in fibroblasts transduced with OSKM and HDAC7wt at the indicated time points. Nuclei stained with DAPI are in blue. E-cad = E-cadherin. Scale = 50 μm. (D) Immunoblot for total HDAC7 in the cytoplasmic fraction versus the nuclear fraction of fibroblasts transduced with OSKM and HDAC7wt at different indicated time points. Histone H3 and GAPDH are loading controls for nuclear fraction and cytoplasmic fraction, respectively. (E) Schematic of HDAC7wt and HDAC7sa (top). Immunofluorescence for total HDAC7 of HEK293T cells transduced with HDAC7sa (bottom). (F) Phase contrast and *Oct4*-GFP$^+$ images of fibroblasts transduced with OSKM and either empty vector (control), HDAC7wt, or HDAC7sa in serum medium + Vc. Scale = 100 μm. (G) Number of GFP$^+$ colonies in fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium (day 18) and serum medium + Vc (day 16). Data is the mean ± standard error of the mean (SEM) (n = 3). The *P* value was calculated using two-tailed unpaired Student's t-test, **P < 0.01. (also hereafter). (H) Phase contrast and *Oct4*-GFP$^+$ images of fibroblasts transduced with OSKM and either empty vector, HDAC4wt, or HDAC4sa in serum medium + Vc. Scale = 100 μm. (I) Number of GFP$^+$ colonies in fibroblasts transduced with OSKM and either empty vector, HDAC4wt, or HDAC4sa in serum medium (day 18) and serum medium + Vc (day 16). Data is the mean ± SEM (n = 3).
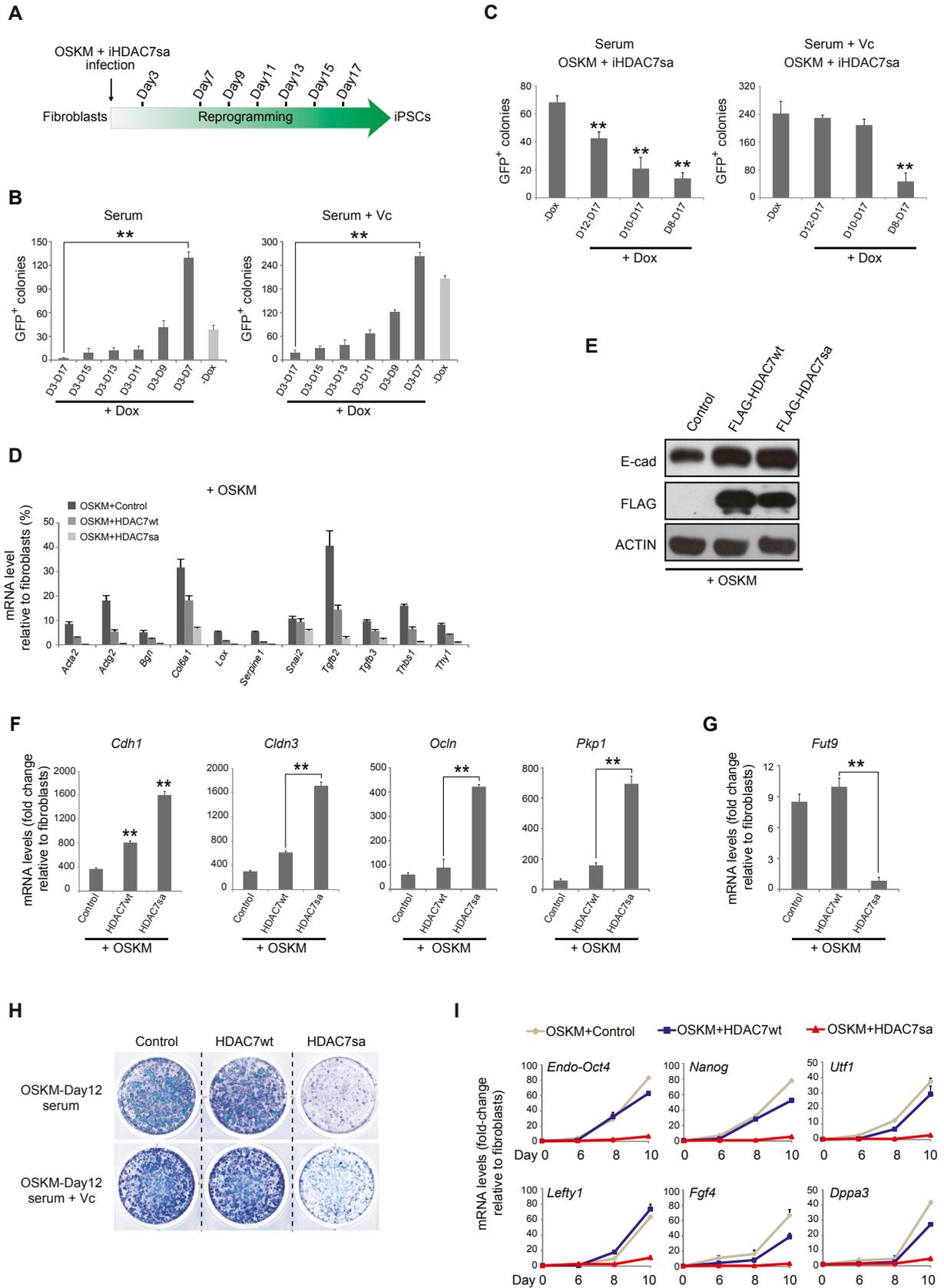
Fig. 2. Dual effect of nuclear HDAC7 in reprogramming (A) Schematic of reprogramming experiments in (B). (B—C) Number of GFP$^+$ colonies at day 18 in fibroblasts transduced with OSKM and iHDAC7sa in serum medium and serum medium + Vc. HDAC7sa expression is induced by adding doxycycline during the indicated time windows. Data is the mean ± SEM (n = 3). (D) RT-qPCR for the indicated genes in fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium at day 6. Data is the mean ± SEM (n = 3). (E) Immunoblot for E-cadherin (E-cad), exogenous HDAC7 (FLAG) and ACTIN, using lysates from fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium at day 7. (F) RT-qPCR for the indicated genes in fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium at day 6. Data is the mean ± SEM (n = 3). (G) RT-qPCR for *Fut9* in fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium at day 8. Data is the mean ± SEM (n = 3). (H) AP activity of fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium and serum medium + Vc at day 12. (I) RT-qPCR for the indicated genes in fibroblasts transduced with OSKM and either empty vector, HDAC7wt, or HDAC7sa in serum medium at the indicated time points. *Endo-Oct4*: endogenous *Oct4*.. Data is the mean ± SEM (n = 3).
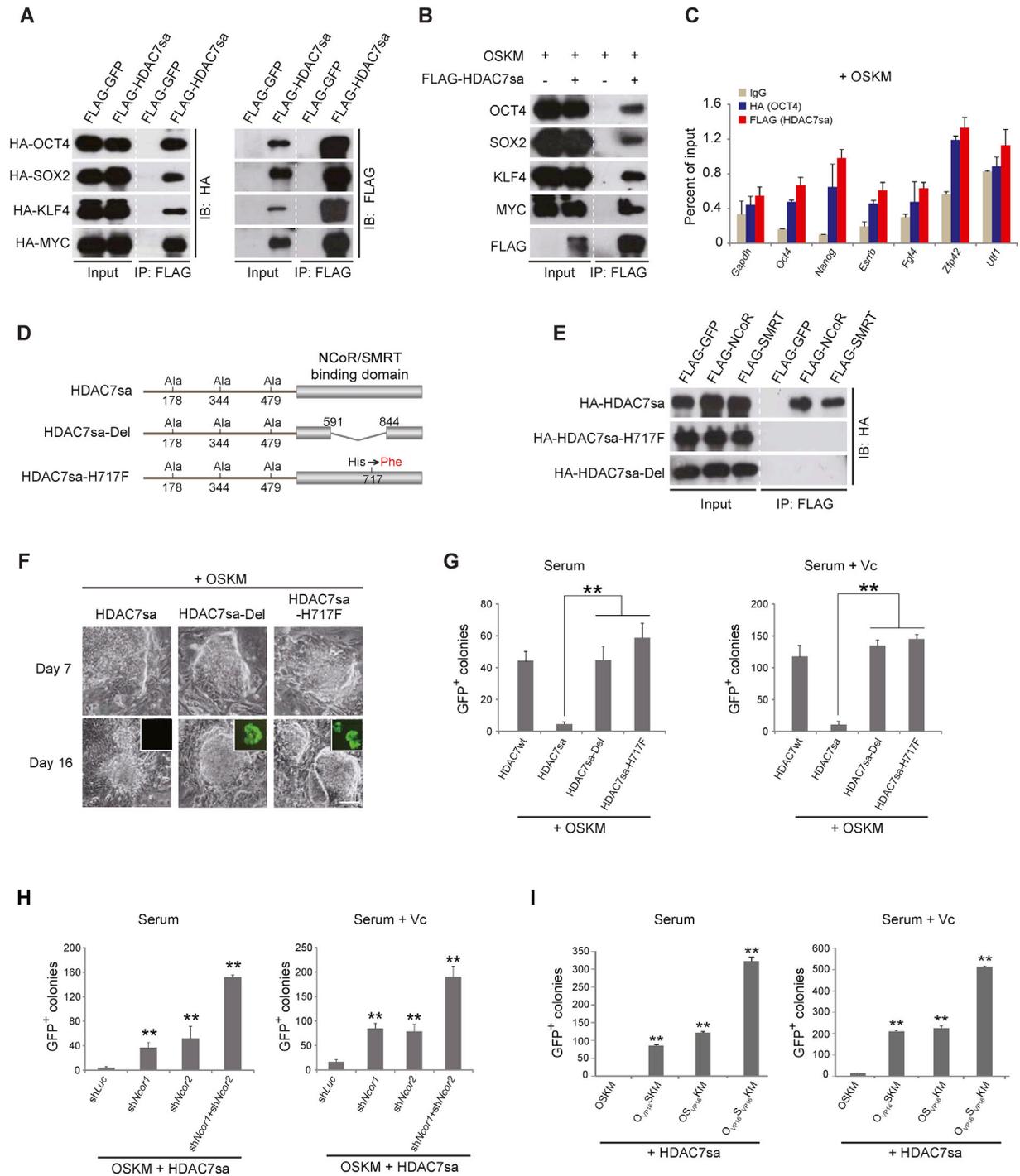
Fig. 3. Nuclear HDAC7 binds to OSKM and recruits NCoR/SMRT co-repressors to derail reprogramming (A) Immunoprecipitation of nuclear extracts from HEK293T cells individually transduced with HA-tagged OCT4, SOX2, KLF4, or c-MYC in combination with FLAG-tagged GFP or HDAC7sa, followed by immunoblot. IP = immunoprecipitation and IB = immunoblot. (B) Immunoprecipitation of nuclear extracts from fibroblasts transduced with OSKM and FLAG-tagged GFP or HDAC7sa at day 8, followed by immunoblot. (C) ChIP of HDAC7 (FLAG) and OCT4 (HA) in fibroblasts transduced with HA-OCT4, SOX2, KLF4, c-MYC, and FLAG-HDAC7sa at day 9, followed by qPCR analysis for the indicated pluripotency genes. Data is the mean ± SEM (n = 4). (D) Schematic depicting HDAC7sa-Del and HDAC7sa-H717F. (E) Immunoprecipitation of nuclear extracts from HEK293T cells transduced with HA-tagged HDAC7sa, HDAC7sa-H717F and HDAC7sa-Del in combination with FLAG-tagged GFP, NCoR or SMRT, followed by immunoblot. (F) Phase contrast and *Oct4*-GFP$^+$ images of fibroblasts transduced with OSKM and HDAC7sa, HDAC7sa-Del, or HDAC7sa-H717F in serum medium + Vc. Scale = 100 μm. (G) Number of GFP$^+$ colonies in fibroblasts transduced with OSKM and HDAC7wt, HDAC7sa, HDAC7sa-Del, or HDAC7sa-H717F in serum medium (day 18) and serum medium + Vc (day 16). Data is the mean ± SEM (n = 3). (H) Number of GFP$^+$ colonies in fibroblasts transduced with OSKM and HDAC7sa, together with shRNAs against *Luciferase* (sh*Luc*), *Ncor1* (sh*Ncor1*), or *Ncor2* (sh*Ncor2*) in serum medium (day 18) and serum medium + Vc (day 16). Data is the mean ± SEM (n = 3) (I) Number of GFP$^+$ colonies in fibroblasts transduced with HDAC7sa and the indicated variants of OSKM in serum medium (day 18) and serum medium + Vc (day 16). Data is the mean ± SEM (n = 3).
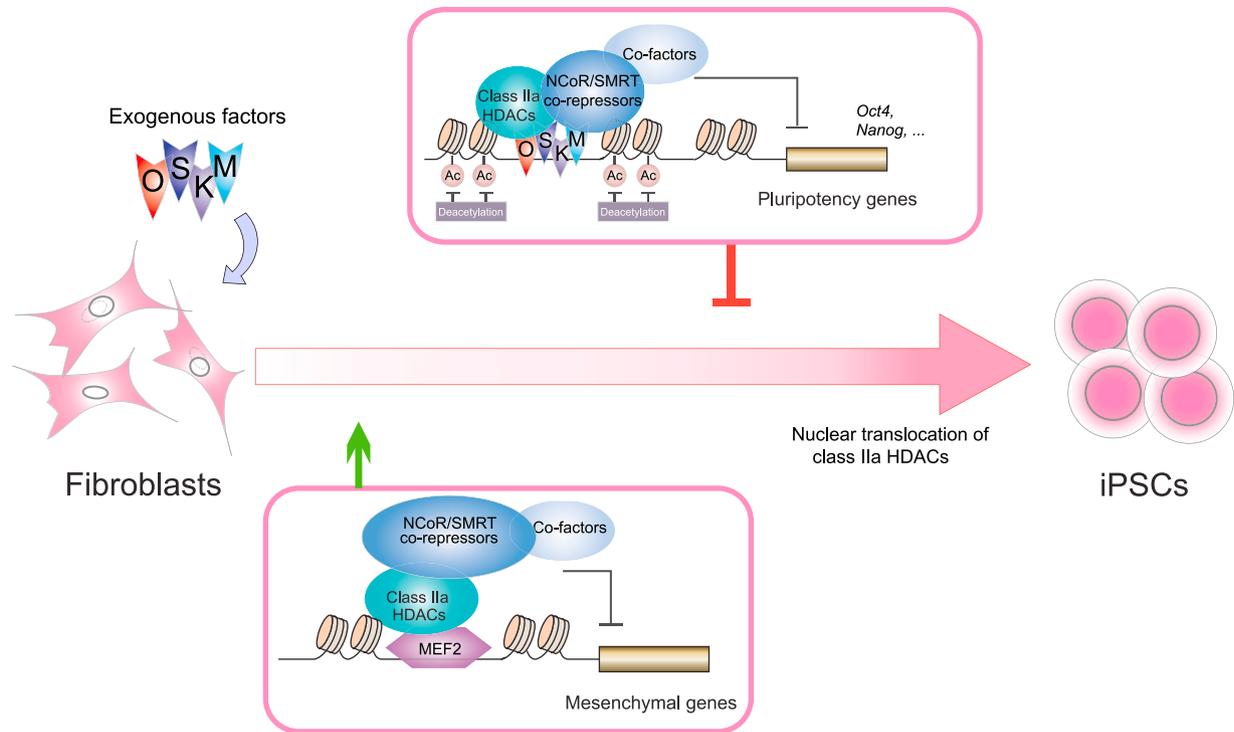
Fig. 4. Schematic depicting the dual function of class IIa HDACs in somatic cell reprogramming. Class IIa HDACs (illustrated by HDAC4 and 7 in this study) bridge NCoR/SMRT co-repressor complex to pluripotency loci through their interaction with exogenous OSKM, thus preventing pluripotency gene reactivation by inducing histone deacetylation. Such mechanistic feature likely regulates the suppression of mesenchymal genes by MEF2 factors in the early phase of reprogramming. NCoR/SMRT may also interact with c-MYC to suppress the somatic cell program independently of class IIa HDACs.[24]

in reprogramming is NCoR/SMRT-dependent, we constructed two HDAC7sa constructs that harbor an additional deletion or amino acid substitution in the NCoR/SMRT interaction domain: HDAC7sa-Del and HDAC7sa-H717F (Fig. 3D). These double HDAC7 mutants retained nuclear localization (Figure S1D), and in agreement with previous studies[36] lost the capacity to interact with NCoR or SMRT (Fig. 3E). Consequently, HDAC7sa-Del and HDAC7sa-H717F also lacked major repressive function, as shown in a MEF2 reporter assay[35] (Figure S1E). We over-expressed these mutants together with OSKM in fibroblasts (Figure S1F) and noticed that GFP[+] colonies appear normally (Fig. 3F and G), as opposed to the blockade observed using HDAC7sa. The boost of reprogramming efficiency caused by these double mutants is not bigger than HDAC7wt likely because they fail to repress MEF2 factors in the early phase of reprogramming.[25] To support these observations, we performed shRNA knockdown of *Ncor1* and *Ncor2* (encoding NCoR and SMRT), observing rescue of the reprogramming blockade caused by HDAC7sa too (Fig. 3H), especially when in combination. Fusion of the transcriptional activator domain of herpes simplex virus VP16 protein to OCT4 (O_{vp16}) and SOX2 (S_{vp16}), which counteracts the detrimental effect of NCoR/SMRT in reprogramming,[37,38] also reversed the blockade by HDAC7sa (Fig. 3I). Overall, these findings indicate that recruitment of NCoR/SMRT co-repressors by class IIa HDACs bound to OSKM on chromatin creates an overwhelming obstacle to pluripotency gene reactivation in reprogramming.

4. Discussion

Here, we have demonstrated that nuclear-cytoplasmic shuttling of class IIa HDACs regulates the efficiency of mouse somatic cell reprogramming (Fig. 4). Nuclear class IIa HDACs enhance reprogramming in the early phase by promoting the MET [25] but derail it later on by blocking pluripotency gene reactivation. It remains to be tested whether the same phenomenon applies to human reprogramming. The fact that ESCs and fully reprogrammed iPSCs display nuclear localization of class IIa HDACs despite cells being pluripotent might be explained by recruitment of additional factors that counteract the detrimental effect. Another possible explanation is that class IIa HDACs in ESCs bind mostly to differentiation rather than pluripotency loci.

In the future, it will be interesting to elucidate the mechanisms controlling class IIa HDACs trafficking in mouse reprogramming. Members from CaMK (Ca^{2+}/calmodulin-dependent protein kinase), MAPK (mitogen-activated protein kinase), SIK (salt inducible kinase), and PKD (protein kinase D) families have been reported to promote class IIa HDAC export from the nucleus through phosphorylation.[39—42] Phosphorylation facilitates the interaction between class IIa HDACs and 14-3-3 proteins. This interaction changes the conformation of HDACs, which either exposes their nuclear export signal or covers their nuclear localization signal.[29,43,44] Thus, genetic or pharmacological manipulation of these kinases may be useful to enhance reprogramming. Because the activity of some of these enzymes is Ca^{2+}-dependent,[45] it would also be important to study whether cellular Ca^{2+} availability changes during reprogramming and how they impact this process.

Notably, the interaction of class IIa HDACs with the Yamanaka factors and MEF2 on chromatin reinforces the idea of macromolecular complexes containing repressors or activators competing in reprogramming. This duality derails pluripotency acquisition to safeguard somatic cell identity.[24,46,47] In this regard, over-expression of wild-type forms of class IIa HDACs enhances OSK but not OSKM reprogramming.[25] Reprogramming with four factors, apart from having a stronger MET phase,[9] has quicker kinetics and this could shift the balance between somatic cell dedifferentiation and blockade of pluripotency gene reactivation induced by nuclear class IIa HDACs towards the latter.

From a wider perspective, our work showcases how developmental pathways influence cell fate transitions. It would be relevant to see whether manipulation of class IIa HDAC nuclear-cytoplasmic trafficking modulates ESC/iPSC differentiation and somatic cell transdifferentiation too.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cr.2018.11.001.

Author contributions

M.A.E., Q.Z., and W.L. conceived the original idea. Z.L., Q.Z., and W.L. conducted the experiments. X.Q., C.B., Z.H., M.Z., Y.H., H.Z., L.W., and Y.L. contributed to the experiments. C.W., G.V., and B.Q. provided relevant advice and/or infrastructural support. M.A.E. supervised the study. M.A.E., W.L., and Z.L. wrote the manuscript. M.A.E. provided funding.

Conflicts of interest

The authors declare no competing interests.

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