RESEARCH ARTICLE

REVISED H3K36me3 and PSIP1/LEDGF associate with several DNA repair proteins, suggesting their role in efficient DNA repair at actively transcribing loci [version 4; peer review: 2 approved, 1 approved with reservations]

Previously titled: "Proteomic analysis of H3K36me3 and PSIP1/p75 (LEDGF) complexes reveal their wider role in DNA repair"

Jayakumar Sundarraj[1,2], Gillian C.A. Taylor[3], Alex von Kriegsheim[3], Madapura M Pradeepa[1,3,4]

[1]Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, E1 2AT, UK
[2]Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Mumbai, 40085, India
[3]MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, EH4 2XU, UK
[4]School of Biological Sciences, University of Essex, Colchester, CO4 3SQ, UK

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Abstract

Background: Trimethylation at histone H3 at lysine 36 (H3K36me3) is associated with expressed gene bodies and recruit proteins implicated in transcription, splicing and DNA repair. PC4 and SF2 interacting protein (PSIP1/LEDGF) is a transcriptional coactivator, possesses an H3K36me3 reader PWWP domain. Alternatively spliced isoforms of PSIP1 binds to H3K36me3 and suggested to function as adaptor proteins to recruit transcriptional modulators, splicing factors and proteins that promote homology-directed repair (HDR), to H3K36me3 chromatin.
Methods: We performed chromatin immunoprecipitation of H3K36me3 followed by quantitative mass spectrometry (qMS) to identify proteins associated with H3K36 trimethylated chromatin in mouse embryonic stem cells (mESCs). We also performed stable isotope labelling with amino acids in cell culture (SILAC) followed by qMS for a longer isoform of PSIP1 (PSIP/p75) and MOF/KAT8 in mESCs and mouse embryonic fibroblasts (MEFs). Furthermore, immunoprecipitation followed by western blotting was performed to validate the qMS data. DNA damage in PSIP1 knockout MEFs was

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1. **Jan De Rijck**, KU Leuven, Leuven, Belgium

2. **Deepak Jha**, Boston Children's Hospital

assayed by a comet assay.
Results: Proteomic analysis shows the association of proteins involved in transcriptional elongation, RNA processing and DNA repair with H3K36me3 chromatin. Furthermore, we show DNA repair proteins like PARP1, gamma H2A.X, XRCC1, DNA ligase 3, SPT16, Topoisomerases and BAZ1B are predominant interacting partners of PSIP /p75. We further validated the association of PSIP/p75 with PARP1, hnRNPU and gamma H2A.X and also demonstrated accumulation of damaged DNA in PSIP1 knockout MEFs.
Conclusions: In contrast to the previously demonstrated role of H3K36me3 and PSIP/p75 in promoting homology-directed repair (HDR), our data support a wider role of H3K36me3 and PSIP1 in maintaining the genome integrity by recruiting proteins involved in DNA damage response pathways to the actively transcribed loci.

Keywords
H3K36me3, PSIP1, LEDGF, SILAC, mass spectrometry, DNA repair, MOF

and Dana-Farber Cancer Institute, Boston, USA

3. **Zeger Debyser**, KU Leuven, Leuven, Belgium

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Corresponding author: Madapura M Pradeepa (p.m.madapura@qmul.ac.uk)

Author roles: Sundarraj J: Investigation, Methodology, Project Administration, Resources, Visualization, Writing – Review & Editing; **Taylor GCA**: Investigation, Writing – Review & Editing; **von Kriegsheim A**: Data Curation, Formal Analysis, Software, Writing – Review & Editing; **Pradeepa MM**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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REVISED **Amendments from Version 3**

We have now added new data showing the presence of PARP1 in the PSIP/p75 complex. Our mass spec data together with the immunoblotting and comet assays show a clear link to PSIP role in DNA repair. Future studies will be needed to understand the clear mechanism through which H3K36 methylated chromatin, PSIP, and its interacting partners contributing to DNA repair.

Any further responses from the reviewers can be found at the end of the article

Introduction

PC4 and SF2 interacting protein (PSIP1) also known as Lens epithelium derived growth factor (LEDGF) is a multifunctional chromatin protein that has been implicated in regulation of homeotic genes, cell survival, cancers and autoimmune diseases. *PSIP1* gene encodes two splice variants – a shorter isoform called p52 and a longer isoform called p75. Both these isoforms possess common domains in their N-terminal regions, namely, PWWP domain and adenine-thymine (AT) hook-like DNA binding domain (Figure 1A). The PWWP domain binds specifically to Histone H3 trimethylated at lysine 36 (H3K36me3); both PSIP1 isoforms and H3K36me3 co-occur at expressed gene bodies (Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014; van Nuland *et al.*, 2013a). The C-terminal integrase binding domain (IBD) is unique to p75, and is shown to interact with HIV integrase (Cherepanov *et al.*, 2003; Ciuffi *et al.*, 2005); the same domain binds to the mixed lineage leukaemia proteins (MLL1, MLL2 and Menin) (Pradeepa *et al.*, 2014; van Nuland *et al.*, 2013b; Yokoyama & Cleary, 2008). Apart from other functions, p75 has also been shown to promote homology directed repair (HDR) by recruiting C-terminal binding protein interacting protein (CtIP) to double stranded breaks (DSB) (Basu *et al.*, 2012; Ciuffi *et al.*, 2005; Daniels *et al.*, 2005; Desfarges & Ciuffi, 2010; Pradeepa *et al.*, 2014; Sutherland *et al.*, 2006).

PSIP/p75 is a multifunctional protein interacts with JPO2 (also known as R1, RAM2 and CDCA7L) (Bartholomeeusen *et al.*, 2007), pogo transposable element with zinc finger domain (PogZ) (Bartholomeeusen *et al.*, 2009), the activator of the S-phase kinase complex (CDC7-ASK) (Hughes *et al.*, 2010), methyl CpG binding protein 2 (MeCP2) (Bartholomeeusen *et al.*, 2009), CtIP (Daugaard *et al.*, 2012), SSRP1 (Lopez *et al.*, 2016), and Spt6 (IWS1) (Tesina *et al.*, 2015), in different genomic location or cellular contexts.

H3K36me3 peptide pulldown followed by SILAC-MS identified PWWP as a putative H3K36me3 reader domain (Vermeulen *et al.*, 2010). Several pieces of evidence support the role of H3K36me3 in the DNA damage response. SETD2 mediated H3K36me3 is shown to recruit PSIP/p75 through PWWP domain to expressed exons (Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014). Upon DNA damage, PSIP1 recruits the repair factors CtIP and RAD51 to facilitate HDR (Aymard *et al.*, 2014;

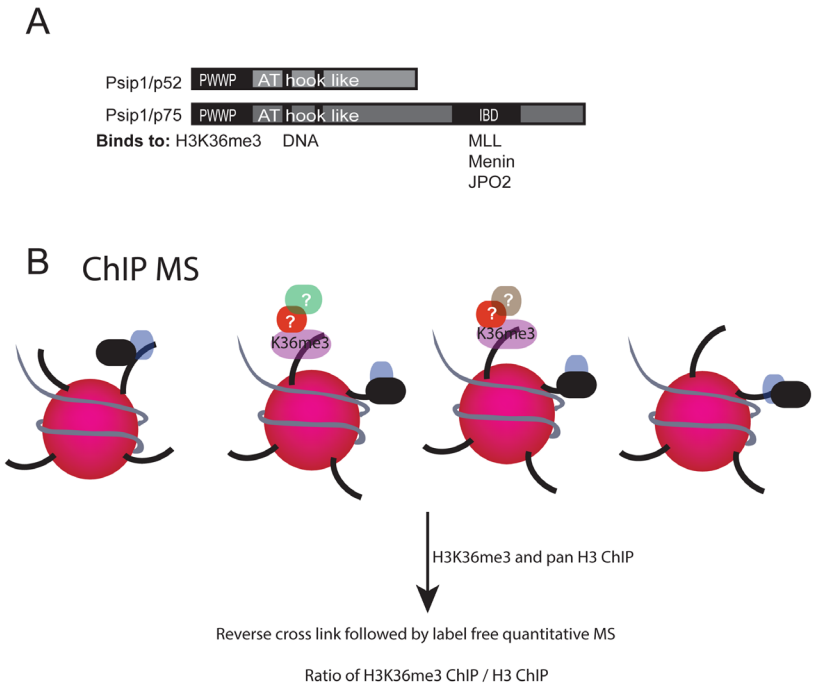

Figure 1. PSIP/p75 domains and its functional interactors. (**A**) Cartoon of PSIP1 p52 and p75 isoforms showing methylated histone binding PWWP domain, DNA binding AT hook domain at the N-terminus and also the integrase binding domain (IBD) at the C-terminus of p75 that interacts with MLL1, Menin and JPO2. (**B**) Illustration showing crosslinked chromatin immunoprecipitation (xChIP) followed by mass spectrometry (MS) to identify proteins associated with histone modifications.

Daugaard *et al.*, 2012; Pfister *et al.*, 2014). All this evidence supports a model in which PSIP1 is anchored to H3K36me3 chromatin at expressed gene bodies through its PWWP domain. Upon DNA damage, chromatin bound PSIP/p75 recruits CtIP and RAD51, which promotes HR repair by efficient resection, and protects these vulnerable regions of the genome from DNA damage. In the absence of SETD2 or H3K36me3, the chromatin association of PSIP1 is reduced, and DNA damage induced recruitment of repair proteins is impaired, leading to reduced resection and HDR. Similarly, another H3K36me3 reader – MRG15 – has been shown to recruit the partner and localiser of BRCA2 (PALB2) complex to undamaged chromatin (Bleuyard *et al.*, 2017). Constitutive association of PALB2 to H3K36me3 chromatin at expressed gene bodies facilitates immediate availability of PALB2 upon DNA damage during active transcription and DNA replication. H3K36me3 is also shown to promote DNA mismatch repair by recruiting the mismatch recognition protein MutSα through its PWWP domain (Li *et al.*, 2013). In contrast to mammalian studies, in budding and fission yeasts, H3K36me3 promotes non homologous end joining (NHEJ) and inhibits HDR (reviewed in (Jha *et al.*, 2014). Similar to yeast studies, H3K36me2, catalysed by SETMAR/Metnase also promotes NHEJ in human cells (Fnu *et al.*, 2011). This suggests a wider and complex role of H3K36 methylation in DNA repair choice and genome stability. Intriguingly, the PWWP domain of PSIP/p75 is also shown to bind H3K36me2 (Zhu *et al.*, 2016) and detected near transcriptional start sites of *Hox* genes suggesting the possibility of binding of PSIP/p75 to H3K36me2 at TSS and to H3K36me3 at the gene bodies (Pradeepa *et al.*, 2014) SETD2, the only enzyme responsible for H3K36 trimethylation is mutated in cancers and is proposed to function as tumor suppressor (Li *et al.*, 2016; Zhu *et al.*, 2014). The methylated H3K36 reader – PSIP1 – is implicated in a variety of cancers (Basu *et al.*, 2016; Daniels *et al.*, 2005; French *et al.*, 2016; Yokoyama & Cleary, 2008) and also implicated in resistance to chemotherapy induced cell death in prostate cancer (Mediavilla-Varela *et al.*, 2009). This suggests that H3K36me3 and PSIP1 play an important role in DNA repair and that dysregulation of this pathway could cause or promote human cancer.

We hypothesised that PSIP/p75 isoform function as an adaptor protein to recruit various proteins involved in DNA repair to H3K36me3 chromatin. We performed formaldehyde cross-linked chromatin immunoprecipitation (ChIP) followed by label-free quantitative mass spectrometry (xChIP-qMS) to identify proteins associated with H3K36me3 chromatin. We find several proteins implicated in transcriptional elongation, RNA processing and DNA repair associated with H3K36me3. Furthermore, SILAC proteomics analysis of endogenous PSIP/p75 complex shows interaction of several DNA repair proteins with PSIP/p75, many of them overlap with proteins enriched in H3K36me3 ChIP. We also detect a higher level of DNA damage in mouse embryonic fibroblasts (MEFs) derived from a *Psip1* knockout mouse (*Psip1* $^{-/-}$) compared to wild type MEFs. We propose a wider role of H3K36me3/PSIP1 axis in maintaining genome integrity and efficient DNA repair at the site of transcription.

Results

xChIP-qMS identifies H3K36me3 associated proteins

H3K36me3 is associated with actively transcribed gene bodies, preferentially at the exons of the expressed genes, suggesting its role in splicing and transcriptional elongation. In order to capture the proteins that transiently and stably associate with H3K36me3, formaldehyde cross linked mouse embryonic stem cells (mESCs) were treated with hypotonic buffer to prepare nuclei, sonicated to obtain soluble chromatin, immuno-precipitated using H3K36me3 and pan H3 antibodies (Figure 1B). This is a useful method to study the proteins that are associated with particular histone modifications. However, since the chromatin is crosslinked and fragmented by sonication to get 100–500bp DNA fragments, many proteins that do not directly bind to H3K36me3 but are bound directly to DNA or to other histone modifications, are also likely to be enriched. Hence, we performed ChIP with the same chromatin using pan-H3 antibodies as control.

Label-free quantitative mass spectrometry analysis of two replicate ChIPs show enrichment of several proteins, implicated in replication, transcription, RNA processing and DNA repair, after anti-H3K36me3 ChIP normalised to anti-pan H3 ChIP (Figure 2 and Dataset 1). Association of RNA processing proteins with H3K36me3 is consistent with the role of H3K36me3 in RNA processing. (Guo *et al.*, 2014; Luco *et al.*, 2010; Pradeepa *et al.*, 2012). Since H3K36me3 is located at expressed gene bodies, it is not surprising that we find several proteins implicated in transcription and transcriptional elongation (Dataset 1). Interestingly, we found 26 proteins that are implicated in DNA repair and are associated with H3K36me3, with >1.5 ratio of H3K36me3 ChIP/H3 ChIP (Figure 2 and Dataset 1). These include known interactors of H3K36me3 – PSIP1, SPT16, SSRP1 and MSH6 (Carvalho *et al.*, 2013; Li *et al.*, 2013; Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014). We detected many PSIP1 peptides mapping to the N-terminal domain that is common to both PSIP1 isoforms. Consistent with our previous work, we also found peptides mapping to the p75 specific C-terminal domain, suggesting the association of both p52 and p75 isoforms of PSIP1 in H3K36me3 chromatin (Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014). Nuck1 (Paralog of RAD51 AP1) protein had the highest H3K36me3/pan-H3 ratio, which was recently shown to promote homologous recombination DNA repair (Maranon *et al.*, 2020).

SILAC immunoprecipitations followed by Mass Spectrometry (SILAC-IP-MS) of PSIP/p75 complex

PSIP1 is a H3K36me3 reader protein, binds to H3K36me3 and localises to expressed gene bodies. The p52 isoform of PSIP1 binds to H3K36me3 and recruits splicing factors to exons of expressed genes (Pradeepa *et al.*, 2012). Similarly, the p75 isoform binds to H3K36me3 and recruits MLL proteins to expressed HOX genes (Pradeepa *et al.*, 2014; van Nuland *et al.*, 2013a; van Nuland *et al.*, 2013b). p75 is also shown to promote HDR by recruiting CtIP and RAD51 to DSBs in a H3K36me3 dependent manner (Aymard *et al.*, 2014; Daugaard *et al.*, 2012; Pfister *et al.*, 2014). In order to comprehensively identify both stable and transient interacting partners of p75, we

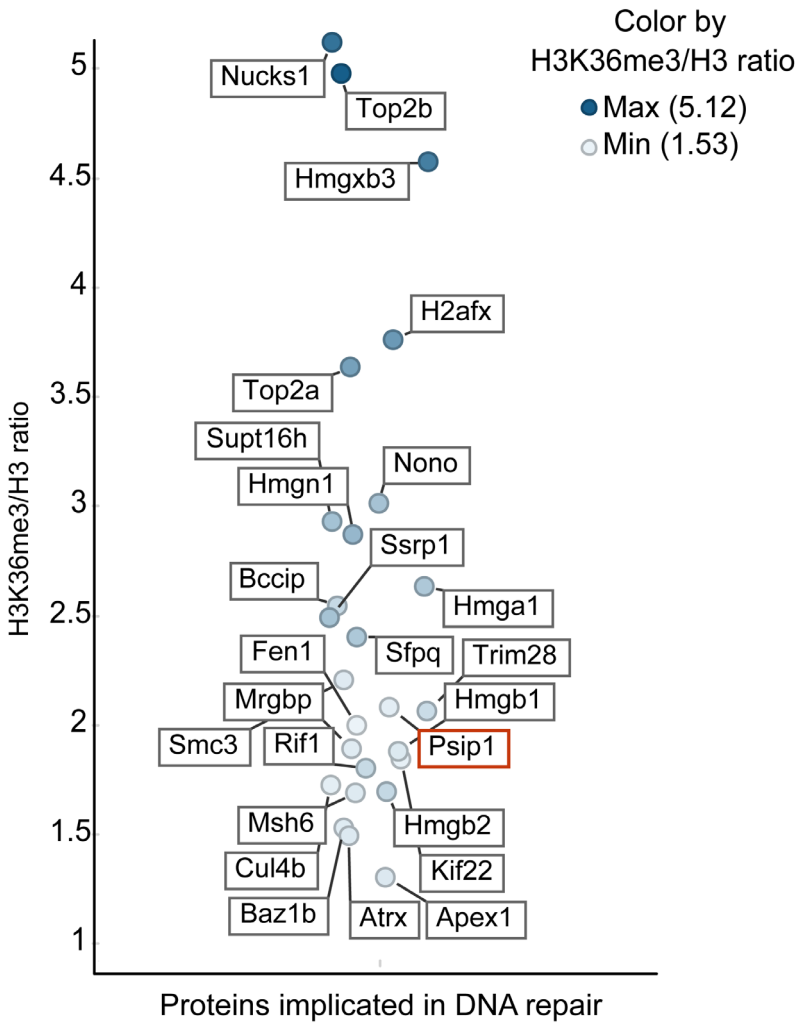

Figure 2. H3K36me3 associated proteins that are implicated in DNA repair. The label-free mass spectrometry quantitative output values assigned to each protein following immunoprecipitation from the mouse embryonic stem cells (mESCs). The list of proteins associated with DNA repair function with the H3K36me3 vs H3 ratio of more than 1.5 (y-axis) are plotted (full list of proteins in Dataset 1). Horizontal scatter was added only to aid visibility of each protein and has no data correlate. The position of PSIP1 protein is highlighted in red.

performed immunoprecipitation (IP) of endogenous p75 protein in cells grown in SILAC media using previously characterised antibodies that specifically pull-down the p75 isoform of PSIP1 (Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014). IP with anti-MOF (KAT8) served as an irrelevant control and rabbit immunoglobulin (IgG) served as a negative control. mESCs and MEFs were first labelled for two weeks in light, medium and heavy SILAC cell culture media, followed by IP-MS with rabbit IgG anti-MOF and anti-PSIP/p75 antibodies, respectively (Figure 3A, Dataset 2 and Dataset 3). The protein enrichment ratio was then calculated to identify proteins that are quantitatively enriched in PSIP/p75 and MOF IP compared to normal rabbit IgG. Similarly, proteins enriched in PSIP/p75 IP normalised to MOF IP was also calculated (Dataset 2 and Dataset 3).

PSIP/p75 interacts with DNA repair proteins

As expected, SILAC-IP-MS with PSIP/p75 and MOF antibodies showed PSIP1 and MOF proteins with the highest SILAC ratio over negative control in respective IPs in both MEFs and mESCs (Table 1; Dataset 2 and Dataset 3). Proteins identified in PSIP/p75 IPs are specific to this isoform of PSIP1, as the antibody used for IP is specific to the c-terminal domain of PSIP/p75, which is absent in the p52 isoform (Figure 1A). Cell division cycle-associated 7 like (CDC7L), one of the known interacting partners of p75, had the second highest ratio in MEFs (Hughes *et al.*, 2010). SILAC ratio for γH2A.X was similar to PSIP1 in mESCs, suggesting the co-occurrence of PSIP1 along with γH2A.X at the nucleosomal level. Interestingly, with the exception of XRCC1 all the other DNA repair proteins found in the p75 complex

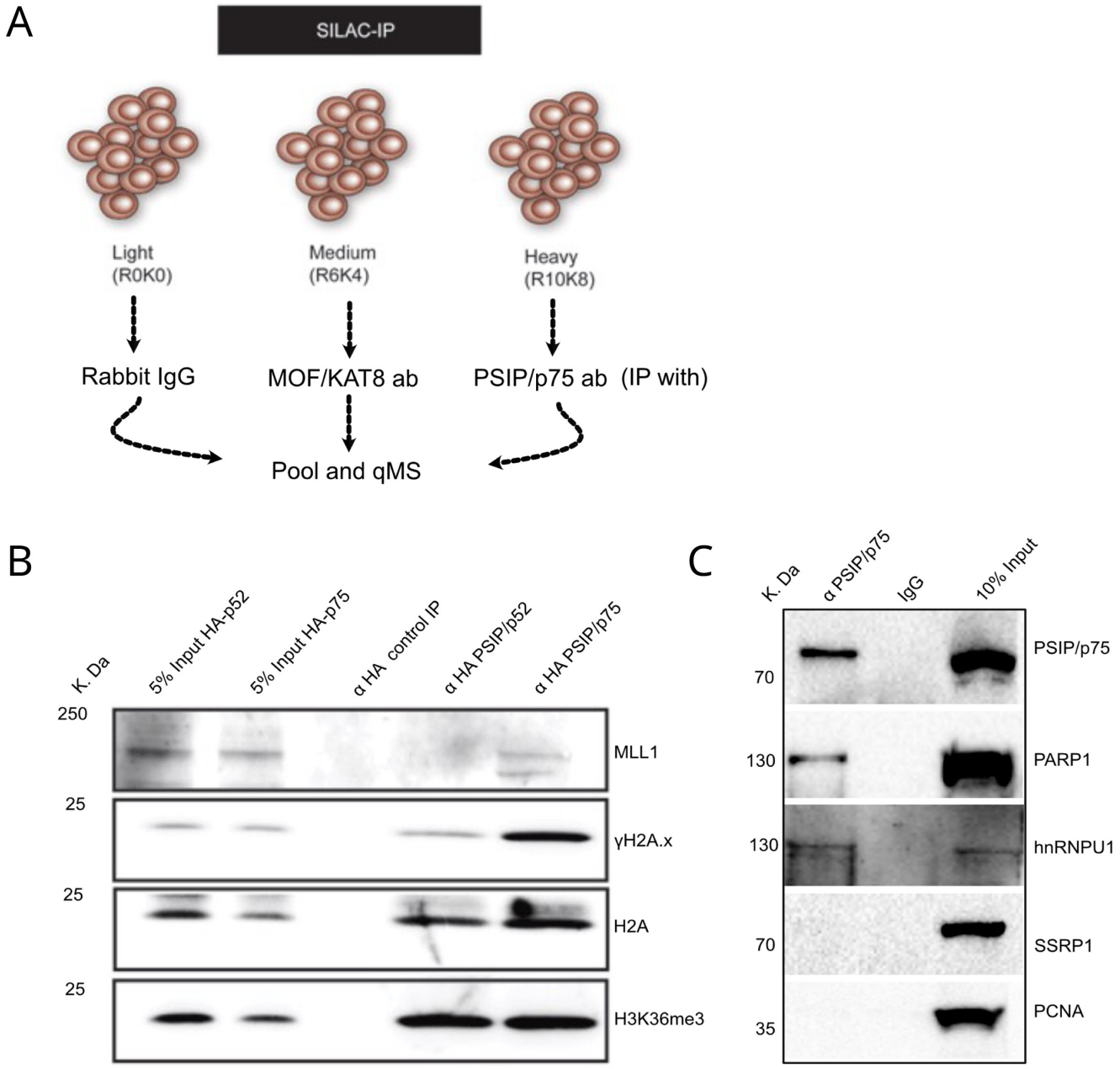

Figure 3. PSIP/p75 role in facilitating DNA repair. (**A**) Illustration showing schematics of SILAC immunoprecipitation for using rabbit IgG, anti-MOF and anti-PSIP/p75 specific antibodies in cells labelled with light (R0K0), medium (R6K4) and heavy (R10K8) SILAC media. (**B**) Western blotting with antibodies recognising MLL1, phosphorylated H2A.X (γH2A.X), H2A and H3K36me3, for HA-tag pulldowns from nuclear extracts of *Psip1* $^{-/-}$ MEFs rescued with HA-p52 and HA-p75. (**C**) Immunoprecipitation using anti- PSIP1/p75 and normal IgG followed by western blotting with PSIP/p75, PARP1, hnRNPU and SSRP antibodies, PCNA antibodies served as a negative control.

were also enriched in H3K36me3 ChIP-MS (Table 1 and Figure 2).

FACT (facilitates chromatin transcription) complex composed two subunits SPT16 (Suppressor of Ty 16) and SSRP1 (Structure-specific recognition protein-1), both were detected in the PSIP/p75 IP. SSRP1 interacts with PWWP domain of PSIP1 (Lopez *et al.*, 2016), which suggesting the functional interplay between PSIP1 and the FACT complex in transcriptional elongation and DNA repair.

Heterogeneous nuclear ribonucleoprotein U (hnRNPU) is detected in MEFs but not mESCs. Although hnRNPU is involved in RNA metabolism, it is known to be involved in promoting

Table 1. List of proteins that associate with PSIP/p75 in mouse embryonic fibroblasts and stem cells. *proteins also found associated with H3K36me3 chromatin (Dataset 1); ND, proteins not detected.

Proteins with higher SILAC ratio	Known function	P75/IgG (MEFs)	P75/IgG (mESCs)
PC4 and SFRS1-interacting protein *	Transcription, alternative splicing Promotes DNA repair	93.5	22
Cell division cycle-associated 7-like	Ser/Thr kinase protein	17.7	6
Histone H2A.X *	DNA damage response	ND	23
Isoform Alpha of DNA ligase 3	NHEJ, BER, SSBR	10.9	ND
PARP1 *	DNA repair	8.7	4
XRCC1	single-strand DNA breaks repair	7.3	ND
FACT complex subunit (SSRP1)	Nucleosome exchange, DNA repair	5.9	ND
FACT complex subunit (SPT16) *	Nucleosome exchange, DNA repair	ND	6.3
Heterogeneous nuclear ribonucleoprotein U Top2 alpha *	DNA Double-Strand Break Signaling and Repair DNA replication & transcription	5.0 9.2	ND ND
Top2 beta *	DNA replication & transcription	ND	2.9
Tyrosine-protein kinase BAZ1B *	H2A.X kinase	3.6	ND

DNA Double-Strand Break Signaling and Repair, hnRNPU proteins promote DNA-end resection and promote ATR dependent signaling and DSB repair by homologous recombination, thereby contributing to cell survival upon exposure to DSB-inducing agents (Polo *et al.*, 2012). Immunoprecipitation done in mESCs detected fewer proteins and also lower SILAC ratio for p75 (Table 1; Dataset 3). Intriguingly, other PSIP1 interacting proteins – MLL1, MLL2, Menin, and/or CtIP – were not detected.

Validation of PSIP/p75 SILAC-MS data

We validated the hits from SILAC-IP-MS for PSIP/p75 by performing immunoprecipitation using PSIP/p75 specific antibody followed by western blotting (Figure 3C), which confirmed the PSIP/p75 interaction with PARP1 and hnRNPU, while SSRP1 was not detected in the p75 IP.

We further validated the interaction of PSIP/p75 with S139 phosphorylated histone H2AX (γH2A.X), MLL1 and H3K36me3, by performing IP with αHA-tag antibodies in *Psip1* knock-out MEFs (*Psip1* $^{-/-}$), which are stably transduced with *HA-Psip1/p75* and *HA-Psip/p52* (Figure 3B) (Pradeepa *et al.*, 2014; Shun *et al.*, 2007). Western blotting of HA IPs with anti-H3K36me3 confirmed the interaction of both p52 and p75 with H3K36me3, which is mediated by a PWWP domain common to both PSIP1 isoforms. Interestingly, we found specific association of a DNA damage marker γH2A.X, with PSIP/p75, but not the p52 isoform. This is consistent with the previously known function of p75 in DNA damage response (Aymard *et al.*, 2014; Daugaard *et al.*, 2012). PSIP/p75 is known to interact with MLL1, but it was not detected in our endogenous p75 IP-MS analysis. However, western blotting with anti-MLL1 shows specific interaction of HA-p75, but not HA-p52 with MLL1 (Figure 3B). These results confirm that although both PSIP1 isoforms are localised to H3K36me3 chromatin, only p75 associates with MLL1, γH2A.X and other DNA repair proteins - consistent with previous reports showing both isoforms of PSIP1 have different protein partners and cellular function (Daugaard *et al.*, 2012; Ge *et al.*, 1998; Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014; Pradeepa *et al.*, 2017).

Higher DNA damage in PSIP1 knockout cells

To examine whether absence of PSIP1 lead to accumulation of unrepaired DNA, we performed comet assay in WT and *Psip1* $^{-/-}$ MEFs, which is a sensitive method to measure DNA damage in individual cells (Olive & Banáth, 2006). In comet assay, cells are mixed with agarose gel matrix, layered on to a slide, subjected to in-gel lysis after solidification, and followed by electrophoresis. When these slides are stained with DNA binding dye and visualized in the microscope, the cells will look like comets. Depending on the extent of DNA damage in a cell, the mobility of the DNA will be more into the tail part of the comet (tail DNA). The Visual scoring of comets showed a significantly higher number of comets with a higher concentration of tail DNA in *Psip1* $^{-/-}$ MEFs compared to WT (Figure 4A) suggesting the presence of unrepaired residual DNA damage in Psip knock-out cells. This data supports our previous observation that human cells depleted of p75 show higher levels of unrepaired DNA compared to control, confirming the higher level of unrepaired DNA in cells lacking PSIP1. This strengthens the evidence for a role for PSIP/p75 in maintaining genomic integrity.

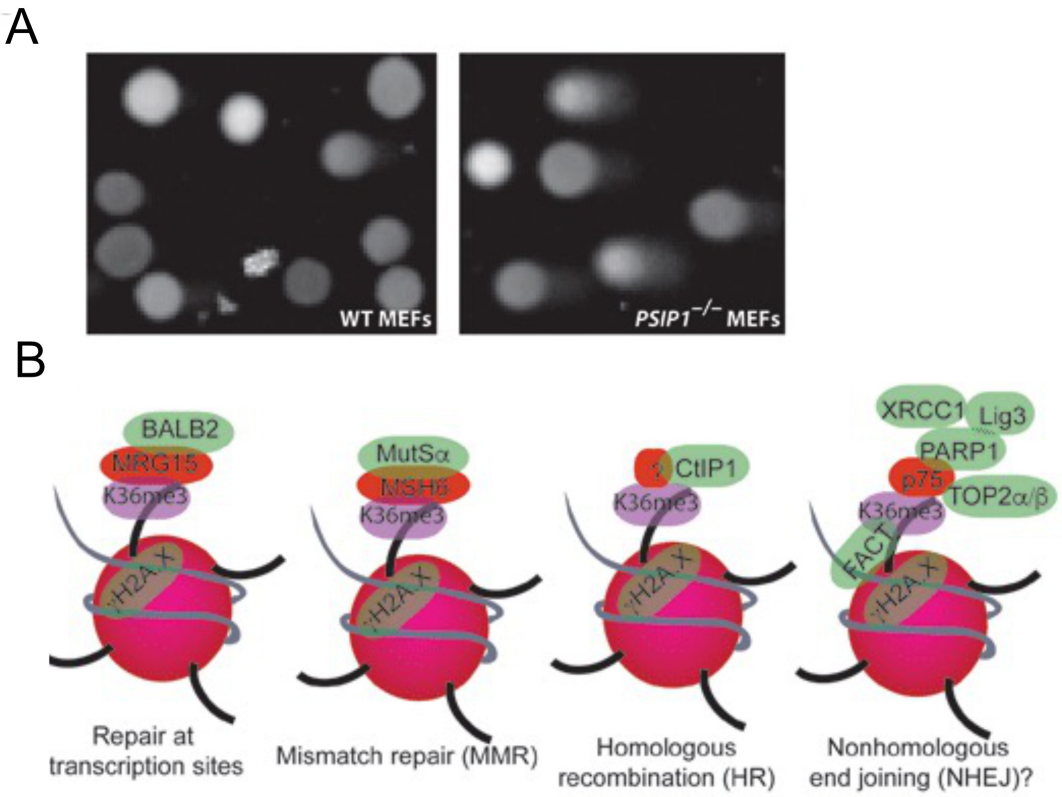

Figure 4. (**A**) Microscopic images of WT and *Psip1* $^{-/-}$ MEFs after Comet assay, a representative image from 16 microscopic fields are shown. (n=2 biological replicates). (**B**) Working model showing various DNA repair proteins that are recruited to H3K36me3 chromatin to modulate repair choice or enhance DNA repair at the site of transcription.

SILAC proteomic analysis of MOF/KAT8 complex

Acetylation of histone H4 at lysine 16 (H4K16ac) and enzymes responsible for H4K16ac – TIP60 (KAT5) and MOF (KAT8) have been implicated with DNA repair (Kumar *et al.*, 2013; Sharma *et al.*, 2010; Tang *et al.*, 2013). Recent work suggests a link between H4K16ac and H3K36me3 in DNA damage response (Li & Wang, 2017). We labelled cells with light, medium and heavy isotope and performed SILAC IP for three different antibodies and were thus able to include anti-MOF IP along with p75 IP and IgG, allowing us to identify the protein partners of MOF along with PSIP/p75 in mESCs and MEFs. MOF IP also acted as an unrelated chromatin protein control that is known to associate with active genes at regulatory elements (Li *et al.*, 2012; Taylor *et al.*, 2013). MOF protein has been shown to be associated with both male-specific lethal (MSL) and non-specific lethal (NSL) complexes (Cai *et al.*, 2010; Li *et al.*, 2009; Mendjan *et al.*, 2006). Like H3K36me3, MOF/MSL complex mediated H4K16ac is enriched at expressed gene bodies. Intriguingly, canonical MSL complex proteins but not NSL or DNA repair proteins were associated in the MOF IP (Dataset 2). There was also no overlap between PSIP and MOF complex proteins which shows the specificity of the PSIP and MOF IP. Although there have been efforts to study protein partners of PSIP and MOF, to our knowledge, this is the first study exploiting the utility of SILAC proteomics to investigate the cellular interactome of these two proteins without using overexpression or epitope tagging approaches. The SILAC immunoprecipitation strategy used here is a very sensitive and powerful means of detecting both transient and stable protein partners of chromatin associated proteins.

Discussion

Immunoprecipitation of nuclear extracts with specific modified peptides followed by SILAC MS led to identification of PWWP domain containing proteins as putative readers of H3K36me3 (Vermeulen *et al.*, 2010). In vitro pulldown of recombinant PSIP-PWWP domain, modified peptide arrays together with ChIPon chip assays confirmed specific interaction of PSIP1 PWWP domain with H3K36me3 (Pradeepa *et al.*, 2012). We now used x-ChIP-qMS, a useful method to identify proteins that interact transiently to histone modifications or chromatin proteins. A similar method was successfully used in *Drosophila* cells to identify MSL associated chromatin proteins and histone modifications (Wang *et al.*, 2013). In this study, we identified proteins associated with H3K36me3 using x-ChIP-qMS. Further studies upon targeted mutations to histone modifying proteins (writers) or domains that recognises these histone modifications (readers) using gene editing methods will aid in validating the specific interaction of H3K36me3 reader proteins.

SETD2 is the only enzyme responsible for majority of H3K36 trimethylation in mammals, and its depletion reduces

H3K36me3 levels, which results in a lower density of FACT subunits SPT16 and SSRP1 (Carvalho *et al.*, 2013). Our data shows that FACT subunits are associated with both H3K36me3 and p75, suggesting the possibility of functional interplay between PSIP/p75 and FACT complex at transcribing gene bodies and in DNA damage signalling response. PSIP/p75 and the SSRP1 complex are suggested to be important for the life cycle of HIV (Lopez *et al.*, 2016). The FACT complex has been shown to facilitate the exchange between H2A.X and H2A (Heo *et al.*, 2008). FACT also promote new H2A.X deposition coupled to repair synthesis and contribute to DNA damage signalling and repair of DNA damage (Piquet *et al.*, 2018). FACT complex promotes incorporation of H2A.X to DNA damage sites, and shown to associate with PARP1 and DNA-PK (Heo *et al.*, 2008). SSRP1 cooperates with PARP1 and XRCC1 to promote single strand DNA break repair. (Gao *et al.*, 2017). Interestingly, PSIP has been shown to function like FACT by allowing RNAPII to overcome the nucleosome-induced barrier to transcription elongation in differentiated cells that no longer express FACT (LeRoy *et al.*, 2019). These evidences suggests FACT and PSIP function together or in same manner to facilitate efficient DNA repair.

Several pieces of evidence have emerged in recent years for the role of histone modifications especially H3K36me3 in DNA repair. Mammalian SETD2 (homolog of Set2) catalyses H3K36me3 at expressed gene bodies in a transcription dependent manner. H3K36me3 in turn recruits PSIP1 (Daugaard *et al.*, 2012; Pradeepa *et al.*, 2012; Pradeepa *et al.*, 2014), MRG15 (Bleuyard *et al.*, 2017; Luco *et al.*, 2010), BS69 (Guo *et al.*, 2014), DNMT3a (Dhayalan *et al.*, 2010) and MSH6 (Li *et al.*, 2013), which modulate transcription, DNA methylation, alternative splicing, and DNA repair choice. A clear association of H3K36me3 with PSIP1 at expressed gene bodies and their association with several DNA repair proteins involved in NHEJ (this work) and HDR (Aymard *et al.*, 2014; Daugaard *et al.*, 2012), suggests wider role of H3K36me3/PSIP1 axis in DNA damage response and genome stability.

Although we have previously shown that both isoforms of PSIP1 bind to H3K36me3 through the common PWWP domain, it is only the p75 isoform of PSIP1 that associates with γH2A.X (Figure 3B). Moreover, most of the previously known p75 interacting proteins are shown to bind to IBD in the C-terminus of p75 (Figure 1A). These data suggest the possibility of other known p75 interacting proteins like PogZ, JPO2, IWS1, MLL and ASK that binds to IBD (Tesina *et al.*, 2015) in DNA repair pathways. It is also possible that PSIP1 binding partners might be interacting with DNA repair proteins leading to enrichment of DNA repair proteins in the H3K36me3 and PSIP1 IP-MS. Future studies involving in vitro reconstitution assays using recombinant proteins will be crucial to demonstrate the direct role of H3K36me/PSIP axis in DNA damage response.

Identification of several DNA repair proteins that interact with PSIP/p75 that are also associated with H3K36me3 suggests that H3K36me3 and PSIP1 have a wider role in DNA repair pathways than previously appreciated. We propose a wide spectrum of roles for SETD2 dependent H3K36me3 and its reader proteins in DNA repair and genome stability than previously suggested (Figure 4B). PSIP/p75 is a stress survival protein, also implicated in various cancers including breast, ovarian, prostate and leukaemia, promote resistance to chemotherapy induced cell death in prostate cancer. Further research is needed for a better understanding of the importance of PSIP1 in promoting DNA repair during stress response, in chemo or radiotherapy induced cell death in cancers.

Methods

Cell lines

Psip1 $^{-/-}$ and its corresponding WT MEFs (Pradeepa *et al.*, 2014; Shun *et al.*, 2007) were a kind gift of Prof. Alan Engelman (Dana-Farber Cancer Institute, USA), and were cultured for two weeks in SILAC DMEM media (Dundee Cell Products), containing 10% fetal bovine serum (HyClone, GE Healthcare) and 1% Pen/Strep (Sigma Aldrich). mESCs (OS25, IGMM biostore) were adapted to grow in DMEM media before they were cultured in SILAC DMEM media. MEFs and mESCs to be used as control (rabbit IgG) in the pulldown were grown under R0K0 media; cells used for MOF IP were cultured in R6K4 media; and cells used for P75 IP were cultured in R10K8 media.

ChIP mass-spectrometry

mESCs were cultured in GMEM as described previously (Pradeepa *et al.*, 2016). Cells were harvested by trypsinization and fixed immediately with 1% formaldehyde (Thermo Fisher, Cat. 28906) (25°C, 10 min) in PBS, and stopped with 0.125M Glycine. Cross linked cells were re-suspended in Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40, Complete Mini EDTA-free protease inhibitor; Roche) for 30 minutes and centrifuged at 228 *g* for 5 minutes at 4°C. Nuclei were resuspended in RIPA buffer (1X PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS (filtered 0.2 -0.45 micron filter unit) + Complete Mini EDTA-free protease inhibitor; Roche) and sonicated using a Bioruptor ® Plus sonication device (Diagenode) at full power for 50 minutes (30 seconds on, 30 seconds off) to produce fragments of 200–500 bp. 10 µg of each antibody was incubated with Protein A Dynabeads (ThermoFisher Scientific, 10001D) in 5 mg/ml bovine serum albumin (BSA) in PBS on a rotating platform at 4°C for two hours. An arbitrary concentration of 200 µg chromatin was incubated with antibody bound Dynabeads on a rotating platform at 4°C for 16 hours. Beads were washed 5 times (5 minutes each) on a rotating platform with cold LiCl wash buffer (100 mM Tris pH 7.5, 500 mM LiCl, 1% NP-40, 1% Sodium deoxycholate) and one time with RT TE buffer.

Antibodies used: 5 µg of rabbit IgG (Santa Cruz sc-2025), Histone H3 (rabbit polyclonal, Abcam, Ab 1791), H3K36me3 (rabbit polyclonal, Abcam, Ab 9050) were used per IP. For analysis by mass spectrometry, beads were washed 3 times with Tris-saline buffer, and excess buffer removed. ChIPed complexes were digested on beads, desalted and analysed on a Q-Exactive plus mass spectrometer, as previously described (Turriziani *et al.*, 2014). Proteins were identified and quantified by MaxLFQ[17] by searching with the MaxQuant version 1.5 against the mouse proteome data base (Uniprot). Modifications included C Carbamylation (fixed) and M oxidation (variable). Bioinformatic analysis was performed with the Perseus software suite.

Immunoprecipitation

10×14-cm dishes of cells were trypsinized and pelleted, resuspended in 5 ml of ice-cold swelling buffer (10 mM Hepes, pH 7.9, 1.5 mM MgCl 2, 10 mM KCl, 0.5 mM DTT and protease inhibitors (Complete, Roche) for 5 min, and cells were broken open to release nuclei using a pre-chilled Dounce homogenizer (20 strokes with a tight pestle). Dounced cells were centrifuged at 228 *g* (2,000 rpm) for 5 min at 4°C to pellet nuclei and other fragments. The supernatant was discarded. The resulting nuclear pellet was resuspended in 5 ml of RIPA buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% Sodium deoxycholate, and protease inhibitors + Benzonase (Novagen, 10 µl/ml), incubated for 30 min on ice, and sonicated briefly on ice (10 × 30 s at full power in bioruptor). Extracts were cleared by centrifugation at 13000 RPM for 10 min at 4°C. Nuclear protein concentrations were measured using a Bradford assay.

Protein A Dynabeads (Life Technologies) were incubated with 5 µg of rabbit IgG (Santa Cruz sc-2025), anti-PSIP/p75 (rabbit polyclonal, Bethyl Laboratories, A300-848A) and anti-MOF (rabbit polyclonal, Bethyl Laboratories A300-992A) in 5% BSA in phosphate buffer saline for two hours, equivalent total protein amounts of extracts were incubated separately with antibodies bound to beads in a rotating platform at 4°C for 30 min. Beads were washed once with RIPA buffer and combined carefully after first wash step. After a further 4 washes, bound proteins were eluted in 4X SDS loading buffer (Life Technologies) with freshly added DTT at 95°C for 5 min. Samples were centrifuged at 11,000 RPM speed for 1 min and supernatant was collect in low binding tube. LC-MS/MS and quantification were carried out by Dundee Cell Products. Briefly, for SDS-PAGE, gel slices per fraction were cut and digested in-gel with trypsin. The purified peptides were then separated (Ultimate U3000, trap-enriched nanoflow LC-system, Dionex), and identified (LTQ Orbitrap XL, Thermoscientific, via nano ES ion source, Proxeon Biosystems). Quantification (MaxQuant, based on 2D centroid of isotope clusters within each SILAC pair) can distinguish between the samples, to give a ratio of protein of interest to IgG. Background proteins would be expected to have a ratio of 1:1 and are therefore disregarded. 10% input nuclear lysate and IPed proteins were separated on Novex 4–20% gels and transferred to PVDF membranes. Western blotting was performed by immunoblotting with PSIP/p75(Bethyl laboratories A300-848A), PARP1(abcam ab191217), hnRNPU (abcam ab10297-50), SSRP1 (Biolegend 609701) and PCNA (Santa Cruz Biotechnology sc7907) antibodies.

HA-pulldown of p52 and p75

PSIP1 knockout MEFs stably rescued with HA-PSIP/p52 and HA-PSIP/p75 were immunoprecipitated with anti-HA antibodies. 5% input lysate and IPed proteins were separated on Novex 4–20% gels and transferred to PVDF membranes. Western blotting was performed by immunoblotting with MLL1 (Active Motif, 61295), γH2A.X (Millipore, 05-636), H2A (Abcam, ab18255) and H3K36me3 (Abcam, ab9050) antibodies.

Comet assay

Comet assays for WT and *Psip1* knockout MEFs were performed using Comet Assay kit (OxiSelect™), according to the manufacturer's instructions.

Data availability

Raw data for this study are available from OSF http://doi.org/10.17605/OSF.IO/UAX7G (Pradeepa, 2017). Dataset 1: ChIP MS data in mESCs, Dataset 2: PSIP/p75 SILAC results in MEFs; Dataset 3: PSIP/p75 SILAC results in mESCs, WT MEFs Comet assay data, PSIP1 KO Comet assay data, and uncropped blots for Figure 3B.

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Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 3

Reviewer Report 06 September 2021

https://doi.org/10.21956/wellcomeopenres.18677.r45684

✓ **Zeger Debyser**
Molecular Virology and Gene Therapy, Department of Pharmacological and Pharmaceutical Sciences, KU Leuven, Leuven, Belgium

In this revised version, the authors address questions asked by referees.

I still have 2 comments:

1. They should explain the results of the comet assay in text or legend for people not familiar with this assay.

2. Also in line with previous comments. Although the data shown suggest an interaction of H3K36me3 and LEDGF/p75 with DNA repair proteins, the analysis used does not discriminate between direct interaction and indirect interaction; e.g. binding partners of LEDGF/p75 may interact with repair proteins. So the claims that LEDGF/p75 by itself is involved in DNA repair should be played down a bit. Therefore a direct interaction with recombinant and purified proteins ought to be shown.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular virology, epigenetics, LEDGF/p75

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 23 October 2017

https://doi.org/10.21956/wellcomeopenres.14001.r26978

?

Jan De Rijck
Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

Since mass spec data are generally known to contain a lot of false positive results, both reviewers requested that the most important mass spec hits should be independently confirmed, by for instance, IP experiments. From all experiments requested by the reviewers, this is the absolute minimum that should be added to the manuscript before approval.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 May 2021

Pradeepa Pradeepa, Queen Mary University of London, London, UK

As we agreed earlier we now have revised the manuscript with new data that supports the interaction of PSIP1 with PARP1 which is one of the key proteins involved in DNA repair.

New data:
We performed immunoprecipitation with PSIP/p75 specific antibody, western blotting of PSIP/p75 IPed complex showed specific enrichment of PARP1 and hnRNPU/SAFA in p75 IP but not SSRP1 one of the subunit of FACT. We also tested several antibodies, unfortunately, many antibodies did not work in our hands. We now believe we have sufficient evidence that supports a wider role of H3K36me3 and PSIP1/p75 in DNA repair pathways

Competing Interests: NA

Reviewer Report 13 October 2017

https://doi.org/10.21956/wellcomeopenres.14001.r26979

Deepak Jha
Division of Pediatric Hematology and Oncology, Boston Children's Hospital and Dana-Farber Cancer Institute, Boston, MA, USA

I do not have any further comments.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epigenetics, Genome Stability, Cell Cycle, Cancer Epigenetics, Hematopoiesis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 25 September 2017

https://doi.org/10.21956/wellcomeopenres.12519.r25938

?

Deepak Jha
Division of Pediatric Hematology and Oncology, Boston Children's Hospital and Dana-Farber Cancer Institute, Boston, MA, USA

The overarching goal of this manuscript by Pradeepa et.al is to identify if PSIP1 isoforms provide a binding platform for DNA repair proteins, which would explain how H3K36me3 functions in DNA repair. They use proteomics to identify, in a somewhat unbiased manner, the interacting partners of H3K36me3 chromatin, and PSIP1/p75. They found a substantial overlap between the binding partners of H3K36me3 and p75. Furthermore, they perform co-IP with Y-H2A.X to validate their Ip-mass spectrometry data. Finally, they show that *Psip1*-/- cells have higher level of unrepaired DNA damage, thereby alluding to a role for *Psip1* -/- in DNA repair.

Specific Comments:

1. As far as I can tell, all of the mass spectrometry experiments have been performed without the induction of any DNA damage. This implies that one is looking at some combination of intrinsic DNA damage associated binding partners, which would be heavily influenced by transcriptional biases (related to gene length, transcriptional frequency and exons), and cell

cycle phases. To directly make a conclusive statement regarding if *PSIP1* acts as a scaffold for recruiting DNA repair proteins, it would be beneficial to monitor the interaction(s) with and without DNA damage. Given the extensive rewiring of cellular signaling after DNA damage, having a more direct readout of differential interacting partners would be more beneficial to refine the various models presented in figure 3d.

2. Figure 3 can be improved by testing some other key interacting partners from table 1. Specifically, components of FACT complex and PARP1 should be tested in co-IP experiments since both of them are key regulators of DNA repair as well.

3. Fig. 3: Additional evidence for unrepaired DNA damage/abrogated DNA damage signaling in *Psip1-/-* should be provided. E.g. Y-H2A.X retention kinetics after DNA damage in *Psip1-/-* cells.
4. Datasets1 and 2 should be labeled in the same manner as the OSF files.

5. The authors state “Interestingly, with the exception of….” and cite Fig.2 for this. They should also refer to table1, alongside Fig.2 to clarify the overlaps.

6. Can the authors phenocopy fig.3c with a PWWP mutant of p75 in order to directly test if H3K36me3- p75 are part of the same mechanism in DNA repair?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epigenetics, Genome Stability, Cell Cycle, Cancer Epigenetics, Hematopoiesis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 September 2017

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Jan De Rijck
Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

Several manuscripts have suggested a role for LEDGF/p75 in transcriptional elongation and DNA repair. Since LEDGF/p75 is known to read H3K36me3, a marker of active chromatin present in the body of genes, and since both H3K36me3 and LEDGF/p75 have been linked to DNA repair it is tempting to speculate that both are part of the same molecular mechanism. Pradeepa *et al.* tried to tackle this question by analysing and comparing the proteome of H3K36me3 and LEDGF/p75.

Comments:

- Recently, LEDGF/p75 has been suggested to interact with H3K36me2 next to H3K36me3 (Zhu *et al* 2016 and Okuda, 2014). Some even claim that this interaction is preferred over H3K36me3. In this regard, LEDGF/p75 can also be found in promotor regions. Although this remains a matter of debate it should be mentioned in the introduction.

- The datasets in the supplemental material are not clear to me. Dataset 1 and 2 are mentioned but not clearly linked to the supplements. I could not find dataset 1.

- Although DNAse is used to overcome DNA bridging in the IP experiments, the xChip experiments are inevitably prone to DNA bridging. One can only claim that these proteins were in the neighbourhood of H3K36me3 marks. This should be clearly indicated in the discussion.

- In the text it is claimed that there is a strong overlay between both datasets in DNA repair proteins. The authors refer to figure 2 to support this statement. However, this figure only mentions H3K36me3 hits. The general overlay between both set could be presented in a better way.

- LEDGF is a major hit in the H3K36me3 xChIP. Looking at the sequenced peptides, can you speculate whether this was p52 or p75?

- Confirmation of some of the important hits by co-immunoprecipitation experiments as presented in figure 3b would strengthen the credibility of the manuscript.

- The fact that LEDGF/p75 has an effect on DNA damage response (figure 3c) is not new. However, neither the present manuscript nor previous manuscripts from other groups could provide a direct link. The authors claim that LEDGF/p75 and not p52 affects DNA repair. As such, there is a good chance that a protein binding to the IBD domain is responsible for this effect. Proteins binding the IBD are characterised by an IBM motif

(Tesina *et al*, 2015).

- Could the authors rescue the DNA repair defect in -/- cells by a mutant LEDGF/p75 protein not able to interact with IBM proteins?

- CDC7/ASK is the only protein in table 1 with an IBM motif. Can HA-LEDGF/p75 rescue DNA repair upon knockdown ofCDC7/ASK?

These data could provide a direct link between LEDGF/p75 and DNA repair.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Oct 2017

Pradeepa Pradeepa, Queen Mary, University of London, UK

We thank Dr. Jan De Rijck for critically reviewing this paper and also suggestions to improve the credibility of the manuscript. Please see our responses below for each of the specific points.

Recently, LEDGF/p75 has been suggested to interact with H3K36me2 next to H3K36me3 (Zhu *et al* 2016 and Okuda, 2014). Some even claim that this interaction is preferred over H3K36me3. In this regard, LEDGF/p75 can also be found in promoter regions. Although this remains a matter of debate it should be mentioned in the introduction.*We have mentioned this is the introduction*

- The datasets in the supplemental material are not clear to me. Dataset 1 and 2 are mentioned but not clearly linked to the supplements. I could not find dataset 1.

We have added the datasets now clearly labeled

- Although DNAse is used to overcome DNA bridging in the IP experiments, the xChip experiments are inevitably prone to DNA bridging. One can only claim that these proteins were in the neighborhood of H3K36me3 marks. This should be clearly indicated in the discussion.

We completely agree with the reviewer's point, hence say these proteins are associated with H3K36me3.

- In the text, it is claimed that there is a strong overlay between both datasets in DNA repair proteins. The authors refer to figure 2 to support this statement. However, this figure only mentions H3K36me3 hits. The general overlay between both sets could be presented in a better way.

We have now referred to Table 1 and Figure 2 and dataset 1

- LEDGF is a major hit in the H3K36me3 xChIP. Looking at the sequenced peptides, can you speculate whether this was p52 or p75?

We have now looked into this and found p75 peptides in the mass spec data. So we cannot rule out the presence of shorter p52 isoform along with the p75 isoform. We didn't find a short peptide that distinguishes between two isoforms. So our data shows both isoforms are likely to present in the H3K36me3 domain.

- Confirmation of some of the important hits by co-immunoprecipitation experiments as presented in figure 3b would strengthen the credibility of the manuscript.

We completely agree with the suggested confirmatory experiments by both reviewers will strengthen the credibility of the manuscript. We will be performing these experiments when we have resources, and we will update the manuscript.

- The fact that LEDGF/p75 has an effect on DNA damage response (figure 3c) is not new. However, neither the present manuscript nor previous manuscripts from other groups could provide a direct link. The authors claim that LEDGF/p75 and not p52 affects DNA repair. As such, there is a good chance that a protein binding to the IBD domain is responsible for this effect. Proteins binding the IBD are characterized by an IBM motif (Tesina *et al*, 2015).

It is an interesting point; we have now discussed this in the manuscript

- Could the authors rescue the DNA repair defect in -/- cells by a mutant LEDGF/p75 protein not able to interact with IBM proteins?

Thanks for suggesting this experiment, we would love to do this experiment in the future, but not for this manuscript

- CDC7/ASK is the only protein in table 1 with an IBM motif. Can HA-LEDGF/p75 rescue DNA repair upon knockdown ofCDC7/ASK?

These data could provide a direct link between LEDGF/p75 and DNA repair.

We appreciate the suggestions to improve the manuscript by both the reviewers. Due to limited resources, we cannot perform the suggested experiments at this time. However, we will perform the suggested experiments in future. We wished to share our data with this publication to wider

readers at the earliest time point.

Competing Interests: No competing interests were disclosed.