British Journal of Cancer www.nature.com/bjc

ARTICLE

SETDB1 tumour suppressor roles in near-haploid mesothelioma involve TP53

Mengting Xu[1,4], Yuqing Tu[1,2,4], Wenhui Bi[1], Meijun Z. Lundberg[3], Isabella Klooster[3], Jonathan A. Fletcher[3] and Wen-Bin Ou[1,3]✉

BACKGROUND: Mutational inactivation of the SETDB1 histone methyltransferase is found in a subset of mesothelioma, particularly in cases with near-haploidy and *TP53* mutations. However, the tumourigenic consequences of SETDB1 inactivation are poorly understood.

METHODS: In this study, we investigated SETDB1 tumour suppressor functions in mesothelioma and explored biologic relationships between SETDB1 and TP53.

RESULTS: Immunoblotting of early passage cultures showed that SETDB1 was undetectable in 7 of 8 near-haploid mesotheliomas whereas SETDB1 expression was retained in each of 13 near-diploid mesotheliomas. TP53 aberrations were present in 5 of 8 near-haploid mesotheliomas compared to 2 of 13 near-diploid mesotheliomas, and BAP1 inactivation was demonstrated only in near-diploid mesotheliomas, indicating that near-haploid and near-diploid mesothelioma have distinct molecular and biologic profiles. Lentiviral SETDB1 restoration in near-haploid mesotheliomas (MESO257 and MESO542) reduced cell viability, colony formation, reactive oxygen species levels, proliferative marker cyclin A expression, and inhibited growth of MESO542 xenografts. The combination of SETDB1 restoration with pemetrexed and/or cisplatin treatment additively inhibited tumour growth in vitro and in vivo. Furthermore, SETDB1 restoration upregulated TP53 expression in MESO542 and MESO257, whereas SETDB1 knockdown inhibited mutant TP53 expression in JMN1B near-haploid mesothelioma cells. Likewise, *TP53* knockdown inhibited SETDB1 expression. Similarly, immunoblotting evaluations of ten near-diploid mesothelioma biopsies and analysis of TCGA expression profiles showed that SETDB1 expression levels paralleled TP53 expression.

CONCLUSION: These findings demonstrate that *SETDB1* inactivation in near-haploid mesothelioma is generally associated with complete loss of SETDB1 protein expression and dysregulates TP53 expression. Targeting SETDB1 pathways could be an effective therapeutic strategy in these often untreatable tumours.

British Journal of Cancer (2023) 129:531–540; https://doi.org/10.1038/s41416-023-02330-x

INTRODUCTION

Mesothelioma is a locally aggressive and highly lethal neoplasm in which the neoplastic proliferation generally originates from pleural or peritoneal mesothelial cells [1, 2]. The main risk factor for mesothelioma continues to be asbestos exposure which is remarkable given that considerable efforts have been made to clear asbestos from the environment starting as early as the 1980s in many countries. Nonetheless, the incidence of mesothelioma so far has not declined because of the extremely long latency of the disease and the persistent environmental exposure to asbestos and other mineral fibres [3]. Mesothelioma histologic subtypes include epithelioid, sarcomatoid, and biphasic [2], of which the sarcomatoid subtype generally has the worst prognosis [4]. Conventional chemotherapies and radiation therapy have limited efficacy against mesothelioma, and substantial improvements in survival will require development of more effective pharmacological interventions.

According to patterns of chromosome losses, mesotheliomas can be classified into near-haploid vs. near-diploid types. Near-haploidy has been observed and studied in subsets of leukaemia [5], but overall seems to be less common in solid tumours. Most mesotheliomas are near-diploid with segmental chromosomal deletions, particularly involving chromosome arms 1p, 3p, 6q, 9p, and 22q [6]. By contrast, fewer than 5% of mesotheliomas are near-haploid, in which the characteristic feature is near genome-wide homozygosity with heterozygosity typically retained only for part or all of chromosomes 5, 7, 15, and 20 [7]. This widespread loss of heterozygosity results from loss of one copy of most chromosomes, resulting in a near-haploid cytogenetic profile. Subsequently, chromosome copy number can be restored by genomic endoreduplication, resulting in a karyotype that appears hyperdiploid but in fact is a doubling of the near-haploid state (two copies of most chromosomes but four copies of the chromosomes that retained heterozygosity). Notably, the chromosomes typically lost in near-haploid mesotheliomas include all the chromosomes in which highly-recurrent segmental deletions are found in near-diploid mesotheliomas [7]. Therefore, near-haploidy accomplishes loss of one copy of the key tumour suppressor genes described to date in mesothelioma.

[1]Zhejiang Provincial Key Laboratory of Silkworm Bioreactor and Biomedicine, College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, China. [2]The First Affiliated Hospital of Soochow University, Suzhou, China. [3]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [4]These authors contributed equally: Mengting Xu, Yuqing Tu. ✉email: ouwenbin@tsinghua.org.cn

Received: 25 July 2022 Revised: 17 May 2023 Accepted: 15 June 2023
Published online: 27 June 2023

Notably, the key tumour suppressor inactivations that occur during development of mesothelioma can sometimes be germline events which create genetic risk for mesothelioma. One mesothelioma genetic risk factor is germline mutational inactivation of the BRCA1-associated protein 1 (BAP1) [8]. *BAP1* mutations also occur as somatic aberrations in many mesotheliomas, as do somatic inactivating mutations and deletions of the *NF2* and *CDKN2A* tumour suppressor genes [9–12], yet no effective targeted therapies exploiting these alterations in mesothelioma have emerged. Other genes recurrently dysregulated by mutations in mesothelioma include SET domain branch type 1 (*SETDB1*), *SETD2*, and *TP53* [7, 11, 13].

SETDB1 is a member of the histone methyltransferase family, which methylates the lysine residue at position 9 of histone H3. SETDB1 coordinates transcriptional repression and silencing of euchromatic genes [14]. During embryogenesis and postnatal development, SETDB1 is required for proviral silencing [15], X-chromosome inactivation [16], myogenesis [17] and differentiation of osteocytes and chondrocytes [18, 19]. Abnormal expression of SETDB1 has been demonstrated in various diseases, including neurologic and psychiatric disorders such as Huntington's disease and schizophrenia, and tumours such as breast cancer [20], lung cancer [21], and prostate cancer [22]. Recent studies demonstrated SETDB1 inactivating mutations in 10% of mesothelioma patients (7/69) by targeted deep sequencing and showed these mutations are associated with near-haploid mesothelioma [7, 11, 13]. These observations suggest that an understanding of SETDB1 dysregulation could lead to new therapeutic options for mesothelioma. However, the tumourigenic consequences of SETDB1 inactivation in mesothelioma are not known. In the current study, SETDB1 inactivation was confirmed as a frequent alteration in near-haploid mesothelioma, but not in near-diploid mesothelioma. SETDB1 restoration resulted in anti-proliferative effects in near-haploid mesothelioma in vitro and in vivo, and SETDB1 regulated TP53 expression in near-haploid mesothelioma. These findings demonstrate that SETDB1 is a key tumour suppressor in near-haploid mesothelioma but not in near-diploid mesothelioma.

MATERIALS AND METHODS

Antibodies, plasmids, and reagents

Monoclonal antibodies to SETDB1 (#2196), SETD2 (#23486), p21 (#2947), Di/Tri-MeH3K9 (#5327), and H3 (#12648) were from Cell Signaling Technology (Beverly, MA), TP53 (sc-126) and BAP1 (sc-28383) were from Santa Cruz Biotechnology (Dallas, Texas), and GAPDH (G8795) was from Sigma-Aldrich (St. Louis, MO). Lentiviral *SETDB1* and *SETDB1-GFP* constructs were purchased from Genecopoeia (Rockville, MD). Lentiviral *TP53 shRNA* constructs were from The RNAi Consortium (TRC), *TP53 shRNA*: 5′-CACCATCCACTACAACTACAT-3′; Lentiviral *SETDB1 shRNA* constructs (TRCN0000148112, TRCN0000276169, and TRCN0000276105) were from Sigma-Aldrich (St. Louis, MO). Pemetrexed and folic acid were purchased from MedChemExpress (Monmouth Junction, NJ). Cisplatin was from Selleck Chemicals (Shanghai, China). PolyJet, Bio-Rad protein assay, and Immobilon Western were from Signagen (Jinan, China), Bio-Rad Laboratories (Hercules, CA), and Millipore Corporation (Billerica, MA), respectively. Puromycin, polybrene and crystal violet were from Sigma-Aldrich (St. Louis, MO).

Mesothelioma cell lines and frozen tissues

Twenty mesothelioma cell cultures were established from surgical materials from previously untreated patients, using standard methods [23, 24]. Loss of heterozygosity and karyotypic aberrations were evaluated by high-density SNP arrays (Cytoscan) and GTG-banding, respectively (Table S1 and Fig. S1). These cell cultures included eight near-haploid mesotheliomas (MESO257, MESO542, MESO136, MESO507, MESO295, MESO963, JMN1B, and MESO59) and 13 near-diploid mesotheliomas (MESO428, MESO924, MESO729, MESO281, MESO381, MESO933, MESO1028, MESO647, MESO863, MESO764, MESO383, MESO188, and MESO296). JMN1B is a subline with enhanced tumourigenicity [25] of the JMN biphasic mesothelioma cell line [26]. Fidelity of each cell culture was corroborated by STR profiling. Cells were regularly screened for mycoplasma contamination using Mycoplasma Stain Assay Kit (Beyotime Biotechnology, Shanghai). All mesothelioma frozen tumour specimens were discarded tissues, obtained from Brigham and Women's Hospital. The Brigham and Women's Hospital and Zhejiang Sci-Tech University Institutional Review Board approved the experiments, and informed consent was obtained from all subjects. Normal mesothelial cells (LP9) were provided by Dr James Rheinwald, having been established from non-neoplastic peritoneum, as described previously [27].

Lentiviral GFP, SETDB1, SETDB1-GFP, TP53 shRNA, or SETDB1 shRNA constructs

Lentivirus preparations were produced by cotransfecting pLKO with *GFP*, expression constructs for *SETDB1* and *SETDB1-GFP*, and *shRNAs* for *TP53* and *SETDB1* with helper virus packaging plasmids pCMVDR8.91 and pMD.G (at a 10:10:1 ratio) into HEK293T cells. Transfections were carried out using PolyJet. Lentiviruses were harvested for 24, 36, 48, and 60 h post transfection. The virus preps were frozen at −80 °C in appropriately sized aliquots for infection.

Cell culture and virus infection

Mesothelioma cell cultures (MESO924, MESO257, MESO542 and JMN1B) were maintained in RPMI 1640 with 10% fetal bovine serum (FBS) supplemented with penicillin/streptomycin and 1% (v/v) L-glutamine. Cells were seeded in six-well plates and lentiviral *SETDB1*, *SETDB1-GFP*, *SETDB1 shRNAs* or *TP53 shRNA* infections were carried out in the presence of 10 µg/mL polybrene. After transduction, cell lines were selected by puromycin (1 µg/mL) for 15, 20, or 30 days. *TP53* knockdown was performed 96 h post SETDB1-GFP infection in MESO542 and MESO257. SETDB1 silencing was performed 96 h post infection with JMN1B.

Protein lysate preparations and immunoblotting

Immunoblotting was performed 96 h post-infection with lentiviral *SETDB1-GFP*, *SETDB1 shRNAs*, or *TP53 shRNA* with or without puromycin selection for 15 or 30 days. Whole-cell lysates from cell lines were prepared using lysis buffer (1% NP-40, 50 mM Tris-HCl pH 8.0, 100 mM sodium fluoride, 30 mM sodium pyrophosphate, 2 mM sodium molybdate, 5 mM EDTA, and 2 mM sodium orthovanadate) containing protease inhibitors (10 mg/mL aprotinin, 10 mg/ml leupeptin, and 1 mM PMSF). Frozen tumour samples were diced into small pieces and homogenised in cold lysis buffer using a Tissue Tearor (Model 398, Biospec Products, Inc. USA), and the cell lysate was then rocked overnight at 4 °C. Lysates were cleared by centrifugation at 15,000 rpm for 30 min at 4 °C and supernatant protein concentrations were determined using a Bio-Rad protein assay. Electrophoresis and immunoblotting were performed as previously described [28]. The hybridisation signals were detected by Immobilon Western and captured using a GE FUJI ImageQuant LAS4000 chemiluminescence imaging system (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). Linear capture quantitation of immunoblotting chemiluminescence signals was performed using an ImageQuant LAS4000. Intensity values were standardised to the Lenti-GFP, Lenti-GFP + H_2O, or pLKO control. Immunoblotting assays were performed by two independent experiments for each cell line.

Immunoprecipitation

Sepharose-protein G beads with mouse polyclonal antibody were used. One mg of protein lysate was preadsorbed for 30 min using 25 µL of protein G beads at 4 °C. Then, 3 µg of primary antibody against TP53 or normal mouse IgG were added to each supernatant and rocked for 2 h at 4 °C. 20 µL protein G beads were added and rocked overnight at 4 °C. The lysates were then spun at 10,000 rpm for 5 min at 4 °C and beads were washed 3 times with 750 µL of IP buffer for 25 min followed once by 750 µL 10 mM Tris-HCl buffer (pH7.6). 20 µL of loading buffer was added to the beads and boiled for 5 min at 95 °C. SETDB1 and TP53 were subsequently evaluated by specific antibody immunoblotting.

Cell viability analysis

Mesothelioma cell lines were plated at 3000 cells/well in a 96-well flat-bottomed plate and cultured for 24 h before treatment with different concentrations of pemetrexed or/and cisplatin in MESO542 and MESO257 with stably expressed SETDB1, as accomplished by transduction with lentiviral *SETDB1*. Proliferation studies were carried out after 3 or 6 days using the CellTiter-Glo luminescent assay from Promega (Madison, WI), and were quantitated using a Promega GloMax 96 Microporous Plate Luminescence Detector. Data were normalised to the control group. These

assays were performed in quadruplicate wells and were averaged from two independent transductions in each cell line.

Colony formation assay

Colony formation assays were performed as described previously with minor modifications [29]. In brief, MESO542 and MESO257 cells or both cell lines with stably expressed SETDB1 were plated at 3000 cells/well in 6-well plates and cultured in RPMI 1640 for 10 days before treatment with pemetrexed. After treatment with pemetrexed for 5 days, the medium was removed, the cells were washed with PBS 2-to-3 times, stained with 0.5% crystal violet in methanol for 20 min, and then washed with distilled water. The stained colonies were photographed and then eluted with 100 µL 33% acetic acid before measuring absorbance at 570 nm. These experiments were performed in duplicate wells and repeated three times.

Xenotransplant murine models

Female adult athymic nude mice (6–8 weeks old) were housed in a specific pathogen-free facility. Mice were injected subcutaneously at bilateral armpits with 1×10^6 MESO542 cells suspended in BD Matrigel expressing SETDB1-GFP ($n = 8$) or Lenti-GFP control ($n = 8$). After 35 days, 4 mice injected with MESO542 Lenti-GFP and 4 mice injected with MESO542 SETDB1-GFP were sacrificed after cervical dislocation. The remaining 8 mice were divided into 4 groups, MESO542-expressing Lenti-GFP ($n = 2$) or SETDB1-GFP ($n = 2$) were treated once daily for another week with pemetrexed (100 mg/kg) by intraperitoneal injection. The others ($2 + 2$) were maintained for a week with water. In the meantime, folic acid was administered in water to reduce toxicity. A week after injection, mice were sacrificed after cervical dislocation and necropsied to evaluate tumour volume and SETDB1 and TP53 signalling. All mouse experiments were conducted in accordance with an Institutional Animal Care and Use Committee.

Genomic–PCR for mutation analysis

DNA was prepared using a QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. In all, 25 ng of total DNA was used for genomic PCR using a Invitrogen Platinum Hot Start PCR Mastermix 2X. PCR-amplified *TP53* or *SETDB1* DNA fragments were purified by exonuclease-SAP approach before sequencing.

RNA preparation and qRT-PCR

Reverse transcription PCR was performed with 1 µg RNA using the PrimeScript™ RT reagent Kit (Takara Bio Inc., Kusatsu, Shiga, Japan). qPCR was performed with TB GreenR Premix Ex Taq™ II (Tli RNaseH Plus) (Takara Bio Inc.) in a reaction volume of 25 µL, using an ABI Prism 7500 real-time PCR detection system (Applied Biosystems Inc., Shanghai). Reactions contained 1 µL cDNA, 400 nM of each primer, and 12.5 µL iQ SYBR green supermix. After 3 min at 95 °C, each of the 40 PCR cycles consisted of denaturation for 10 s at 95 °C, hybridisation of primers and SYBR green, and DNA synthesis for 1 min at 60 °C. The qRT-PCR assays for *SETDB1* and *TP53* were performed using the following primers: *SETDB1* sense: 5′-GACGGGAGAGGACAAAAGCA-3′ and anti-sense: 5′-AGTTCCTCAACCACTGCCTG-3′; *TP53* sense: 5′-TAACAGTTCCTG CATGGGCGGC-3′ and antisense: 5′-AGGACAGGCACAAACACGCACC-3′; *GAPDH* sense: 5′-GAAGGTGAAGGTCGGAGTCAAC-3′ and anti-sense: 5′-TGG AAGATGGTGATGGGATTTC-3′. All primers were obtained from Invitrogen. The comparative Ct (cycle threshold) method was used to determine RNA expression fold differences in mesothelioma cell lines after treatment with lentiviral *SETDB1* or *TP53* shRNAs. The data points (run in triplicate assays) were normalised to *GAPDH*.

Statistical analysis

T tests were performed on data from cells treated with control *Lenti-GFP*, *pLKO*, or H_2O, as well as cells treated with *SETDB1*, *SETDB1-GFP*, *TP53 shRNA*, cisplatin, or pemetrexed. Statistically significant differences between the controls and treatment by Graphpad Prism5.0 were defined as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

RESULTS

SETDB1 expression is undetectable in most near-haploid mesotheliomas whereas expression is retained in near-diploid mesotheliomas

Immunoblotting showed complete loss of SETDB1 expression in seven of eight near-haploid mesothelioma cell cultures (MESO257, MESO542, MESO136, MESO507, MESO295, MESO963, and MESO59) whereas SETDB1 was expressed in each of 13 near-diploid mesothelioma cell cultures (MESO428, MESO924, MESO729, MESO281, MESO381, MESO933, MESO1028, MESO647, MESO863, MESO764, MESO383, MESO188, and MESO296) (Fig. 1a). *SETDB1* hemizygous or homozygous nonsense or frameshift mutations were demonstrated in three of the near-haploid cases with SETDB1 expression loss (MESO257 R616X; MESO136 E724X; and MESO542 pQ811EfsTer11).

SETDB1 transcript expression comparisons between mesothelioma and other cancer types were performed using TCGA profiling data [7]. These comparisons showed that *SETDB1* expression across 87 mesotheliomas was comparable to most other cancer types, including lung carcinomas (Fig. 1b). This is consistent with the lack of SETDB1 aberrations in near-diploid mesothelioma (Fig. 1a), which account for at least 95% of mesotheliomas. Although mesotheliomas with lower *SETDB1* expression showed a trend towards poorer overall survival, this association was not statistically significant (Fig. 1c).

Restoration of SETDB1 inhibits cell proliferation of near-haploid mesothelioma

SETDB1 expression was restored in MESO542 and MESO257 near-haploid mesotheliomas by lentiviral infection with *SETDB1* and *SETDB1-GFP* followed by puromycin selection for 30 days (Fig. 2). Immunoblotting demonstrated that this SETDB1 restoration induced methylation of the lysine residue at position 9 of histone H3 (H3K9), namely increased Di/Tri-Methyl-H3K9, compared to control arms with infection using *Lenti-GFP* (Fig. 2a). JMN1B cells served as a positive control for SETDB1 and Di/Tri-Methyl-H3K9 expression.

Anti-proliferative effects of SETDB1 restoration were evaluated by cell viability, colony formation assays, ROS generation, and cyclin A expression in MESO542 and MESO257 in vitro and in vivo (Fig. 2b–h). SETDB1 restoration resulted in a 25%–40% reduction in cell viability in both of these near-haploid mesotheliomas, as compared with the same cells after infection with the *Lenti-GFP* control (Fig. 2b). Likewise, SETDB1 restoration inhibited colony formation (Fig. 2c), resulting in 10-to-26% decreased colony formation in MESO257 and 33-to-40% decreased colony formation in MESO542 (Fig. 2d). Further, SETDB1 restoration inhibited growth of MESO542 xenografts in mice, with reductions in xenograft size and weight compared to MESO542 xenografts containing only the *Lenti-GFP* control construct (Fig. 2e, f). SETDB1 restoration also inhibited the mesothelioma proliferation biomarker, cyclin A, in the MESO542 xenografts (Fig. 2g). Additionally, SETDB1 restoration inhibited ROS generation in MESO542 and MESO257 (Fig. 2h).

Pemetrexed and/or cisplatin confer additive anti-proliferative effects after SETDB1 restoration in near-haploid mesothelioma

Additive inhibition cell viability was demonstrated with the combination of pemetrexed treatment and SETDB1 restoration in near-haploid mesothelioma cell lines (Fig. 3a, e). Whereas pemetrexed (0.5 µM) treatment of *Lenti-GFP* control cells demonstrated 20% and 70% reductions of viability in MESO542 and MESO257, respectively, the same treatment after lentiviral SETDB1 restoration resulted in 40% and 80% reductions in viability, respectively, in MESO542 and MESO257 (Fig. 3a).

Additive anti-proliferative effects were further evaluated by colony formation assays. Colonies of MESO542 and MESO257 cells treated with pemetrexed or infected with lentiviral *SETDB1* were fewer when compared with H_2O or *Lenti-GFP*-treated cells (Fig. 3b, c). Pemetrexed treatment in combination with SETDB1 restoration further decreased the number of colonies in MESO542 and MESO257, as compared with either intervention alone (Fig. 3b, c). Relative to a Lenti-GFP control, MESO542 and MESO257 colony formation decreased by 10% in cells infected with SETDB1, by 50%

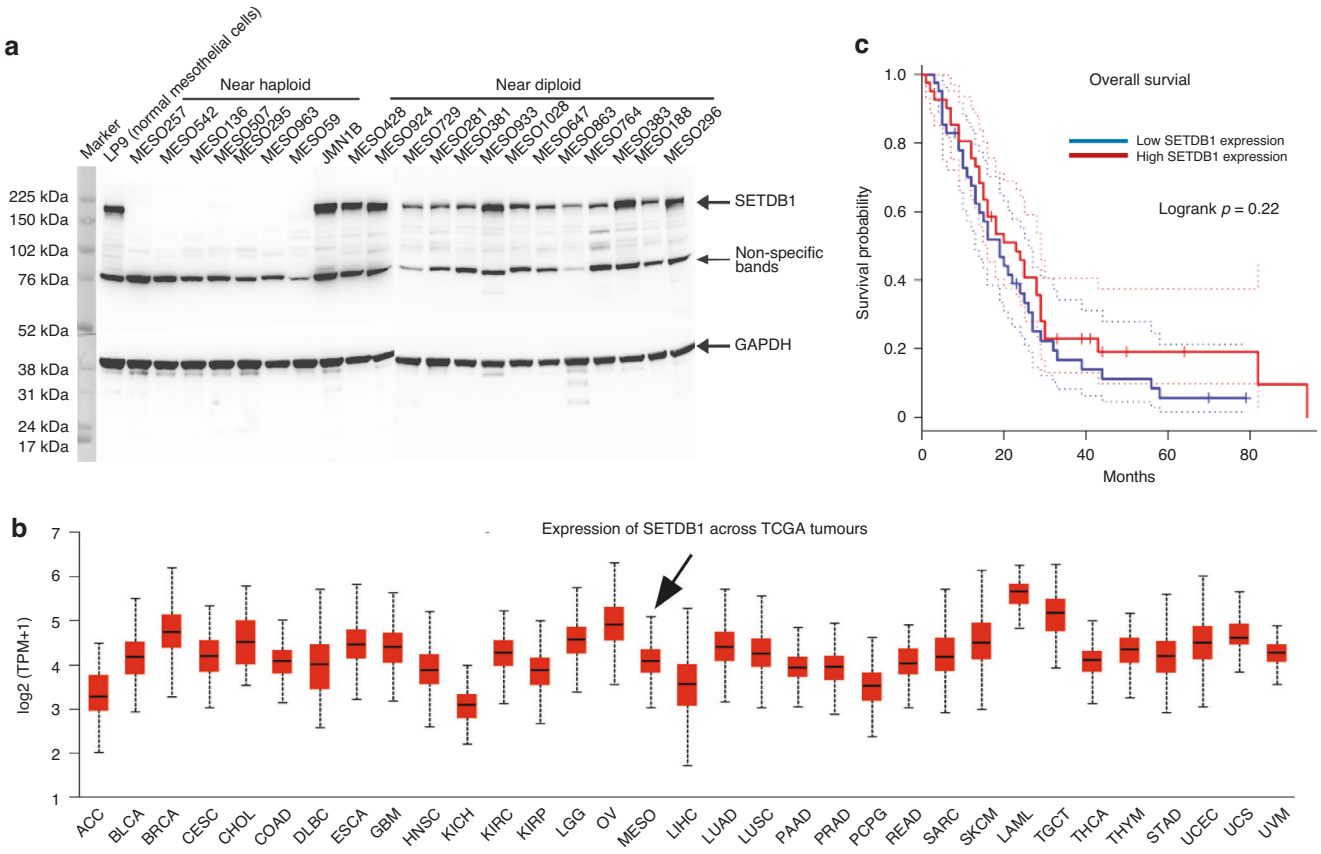

Fig. 1 SETDB1 expression in mesothelioma. a Immunoblotting evaluation of SETDB1 expression in eight near-haploid mesothelioma cell lines (MESO257, MESO542, MESO136, MESO507, MESO295, MESO963, MESO59, and JMN1B) and in thirteen near-diploid mesothelioma cell lines (MESO428, MESO924, MESO729, MESO281, MESO381, MESO933, MESO1028, MESO647, MESO863, MESO764, MESO383, MESO188, and MESO296). LP9 is a non-neoplastic mesothelial cell control. GAPDH stain is a loading control. **b** TCGA gene expression profiling data analysis for 87 mesotheliomas shows that SETDB1 expression is lower or comparable in mesothelioma patient samples compared to other tumour types. **c** Survival analysis of TCGA mesothelioma dataset demonstrates that low SETDB1 expression level has a trend towards poor overall survival (but is not statistically significant) in mesothelioma patients.

and 40% in cells treated with pemetrexed, and by 75% and 50% in cells treated with pemetrexed in combination with SETDB1 restoration (Fig. 3c).

In a MESO542 mouse xenograft model, pemetrexed treatment in combination with SETDB1 restoration resulted in a greater decrease in tumour size and weight compared to either intervention alone (Figs. 3d, e and S2). Likewise, additive inhibition of the cyclin A proliferation marker in MESO542 xenografts resulted from the combination of SETDB1 restoration and pemetrexed (Fig. 3f).

Additive anti-proliferative effects were also evaluated after treatment with cisplatin in near-haploid mesothelioma with SETDB1 restoration (Fig. 3g). Treatment with cisplatin showed anti-proliferative effects in MESO542 and MESO257 with Lenti-GFP control infection in a dose-dependent manner. Whereas cisplatin (2.5 μM) treatment resulted in 50% and 20% reduction in viability for MESO542 and MESO257, respectively, compared with a H_2O control (Fig. 3g), the combination of cisplatin and SETDB1 restoration resulted in 60% and 40% reduction in viability in these near-haploid mesotheliomas. Further, a combination of pemetrexed (0.25 μM), cisplatin (2.5 μM) and SETDB1 restoration resulted in 65% and 80% reduction in viability for MESO542 and MESO257, respectively (Fig. 3g).

SETDB1 is positively associated with TP53 expression

Our previous studies demonstrate that multiple pathways regulate TP53 expression in mesotheliomas [4, 30, 31], and other studies demonstrate that SETDB1 in liver cancer cells regulates TP53 expression [32]. Thus, we evaluated TP53 expression after stable SETDB1 restoration in MESO542 and MESO257 (Fig. 4a). Immunoblotting showed that SETDB1 restoration up-regulated TP53 expression in both cell lines (Fig. 4a). Further, *SETDB1* shRNA knockdown inhibited expression of mutant TP53 protein and mRNA in the one near-haploid mesothelioma (JMN1B) that retains SETDB1 expression (Fig. 4b, c).

We next evaluated associations between SETDB1, TP53, p21, SETD2, and BAP1 expression in near-haploid mesothelioma and near-diploid mesothelioma cells (Fig. 4d). TP53 and p21 expression was abnormal, compared to non-neoplastic LP9 mesothelial cells, in five of eight near-haploid mesotheliomas and two of 13 near-diploid mesotheliomas. TP53 was over-expressed in two near-haploid mesotheliomas (MESO507 and JMN1B) and one near-diploid mesothelioma (MESO764), whereas TP53 expression was down-regulated in three near-haploid mesotheliomas (MESO136, MESO963, and MESO59) and one near-diploid mesothelioma (MESO383). Genomic sequencing demonstrated *TP53* point mutations (Table S2) in four of the near-haploid mesotheliomas with aberrant TP53 protein expression, and p21 expression was substantially reduced in all mesotheliomas with aberrant TP53 expression (Fig. 4d). BAP1 was expressed at levels comparable to that in LP9 cells in each of 8 near-haploid mesotheliomas but was lost in 6 of 13 near-diploid mesotheliomas (MESO281, MESO933, MESO1028, MESO647, MESO764, and MESO383). SETD2 expression was

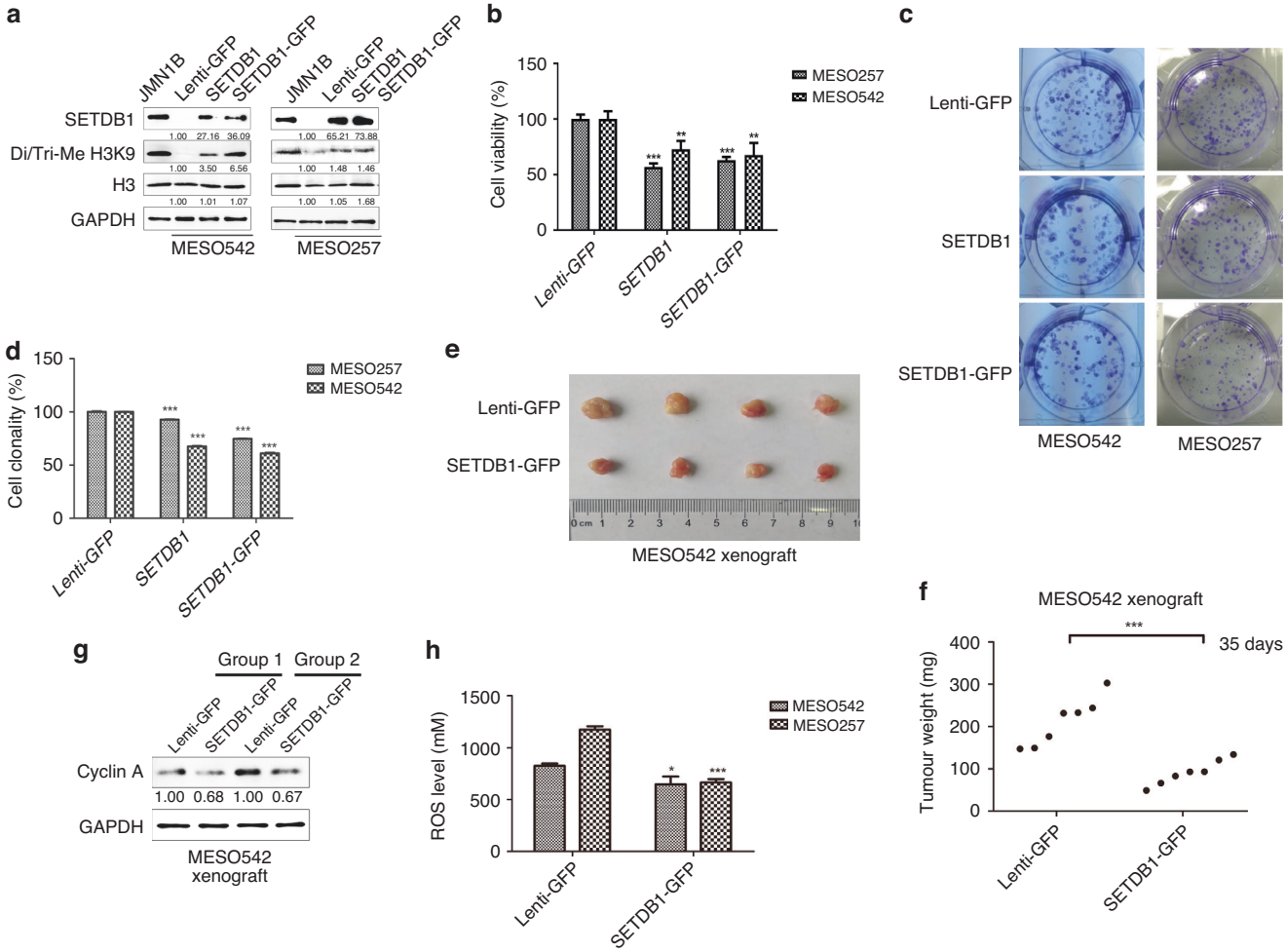

Fig. 2 Evaluations of near-haploid mesothelioma cultures (MESO257 and MESO542) after lentiviral mediated SETDB1 restoration. **a** Immunoblotting evaluation of SETDB1 impact on Di/Tri-methyl H3K9 expression, 30 days post infection and selection (1 μg/mL puromycin). GAPDH stain is a loading control. Near-haploid mesothelioma cell line JMN1B is a SETDB1-retaining comparator. **b** Cell viability was evaluated by CellTiter Glo® ATP-based luminescence assay, 30 days post infection and selection (1 μg/mL puromycin). Data were normalised to Lenti-GFP control and represent mean values (±s.d.) from quadruplicate cultures, averaged from two independent experiments for each cell line. Statistically significant differences between Lenti-GFP control and SETDB1/SETDB1-GFP construct infections are presented as $^{**}p < 0.01$ and $^{***}p < 0.001$. **c** Colony growth assays were performed 10 days after infection with SETDB1 or SETDB1-GFP constructs and selection (1 μg/mL puromycin) for 30 days. Colony growth experiments were performed in triplicate. SETDB1 restoration led to a greater reduction in colony formation in MESO257 and MESO542 than Lenti-GFP control. Scale bar: 3.5 mm. **d** Quantitation of MESO257 and MESO542 cell colony growth after SETDB1 restoration. Statistically significant differences between Lenti-GFP control and SETDB1/SETDB1-GFP infections are presented as $^{***}p < 0.001$. **e** Lentiviral-mediated SETDB1 restoration inhibited mesothelioma xenograft growth **f** Lentiviral-mediated SETDB1 restoration inhibited mesothelioma xenograft weight. Statistically significant differences between Lenti-GFP control and SETDB1-GFP infections are presented as $^{***}p < 0.001$. **g** Immunoblotting evaluation of proliferation marker cyclin A in near-haploid mesothelioma (MESO542: Lenti-GFP and SETDB1-GFP restoration) xenografts. GAPDH stain is a loading control. **h** ROS levels were analysed in MESO542 and MESO257 after SETDB1 restoration. Statistically significant differences between Lenti-GFP control and SETDB1-GFP infections are presented as $^{*}p < 0.05$ and $^{***}p < 0.001$.

comparable between near-haploid mesotheliomas and near-diploid mesotheliomas (Fig. 4d).

Immunoblotting demonstrated that SETDB1 expression levels paralleled TP53 expression in 10 mesothelioma tissue samples (Fig. 4e). Comparisons of *SETDB1* expression and *TP53* mutation status were performed with the published TCGA mesothelioma expression profiles from 83 patients [7]. This confirmed that *SETDB1* expression was reduced in *TP53*-mutant mesotheliomas ($p < 0.021417$), of which 4 were near-haploid (Fig. 4f).

TP53 regulates SETDB1 expression in near-haploid mesothelioma

To further characterise relationships between TP53 and SETDB1 expression in near-haploid mesothelioma, we evaluated the impact of lentiviral-mediated *TP53* knockdown in MESO542 and MESO257 after stable expression of a SETDB1 construct (Fig. 5a, b). As compared with Lenti-GFP control, SETDB1 restoration increased expression of Di/Tri-Methyl-H3K9 and TP53 in MESO542 and MESO257 (Fig. 5a, b). *TP53 shRNA* knockdown down-regulated expression of SETDB1 protein and mRNA, accompanied by reduced H3K9 2/3 methylation in both cell lines (Fig. 5a, b).

The association of SETDB1 and TP53 was further evaluated in MESO542 xenograft tumour tissues (Fig. 5c, d). Immunoblotting showed that SETDB1 restoration induced expression of Di/Tri-methyl H3K9, and also resulted in TP53 upregulation (Fig. 5c, d). Di/Tri-Methyl H3K9 and TP53 expression were further induced in SETDB1 restoration mouse xenografts by pemetrexed treatment, but not in Lenti-GFP and null SETDB1 controls (Fig. 5d).

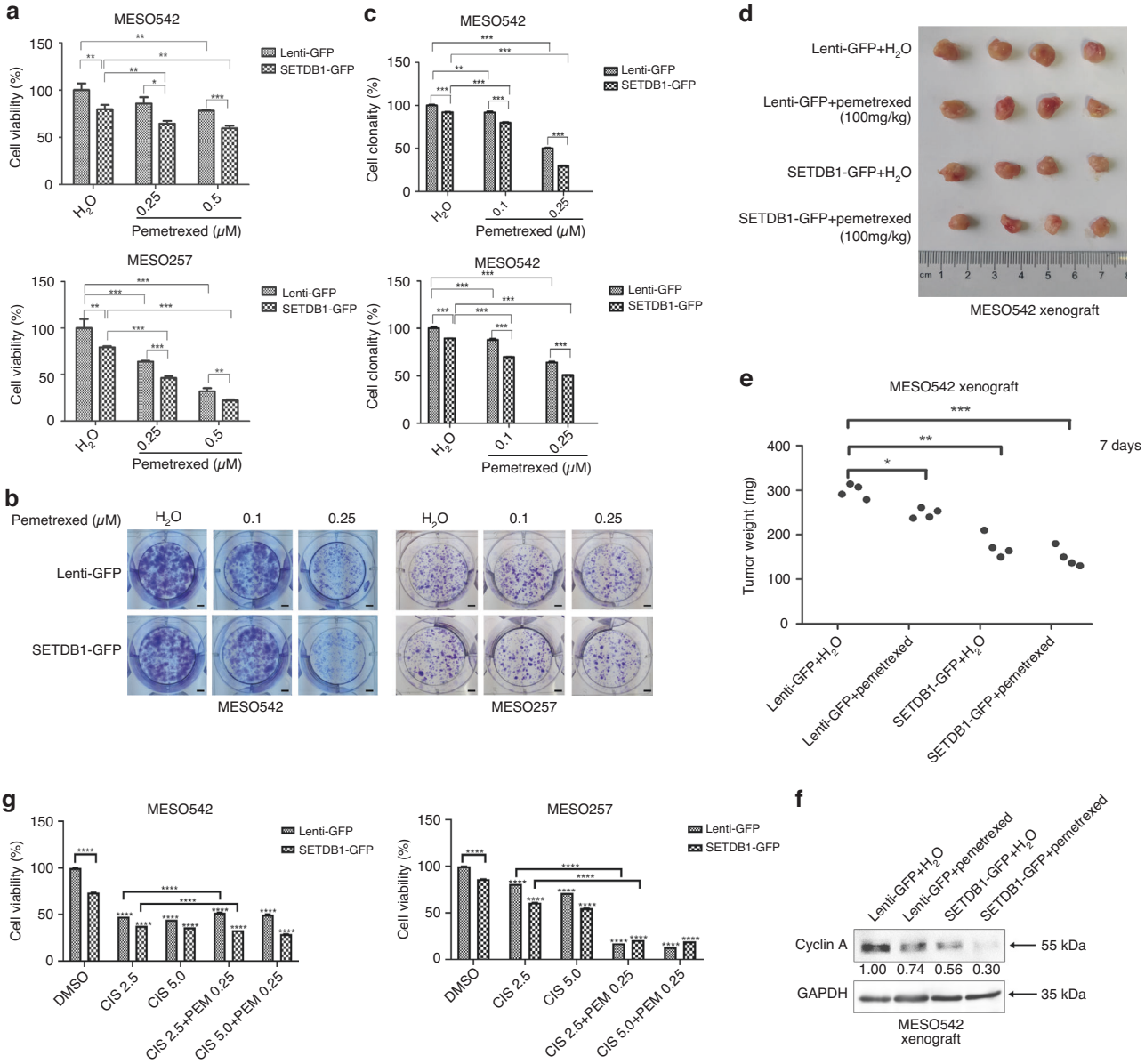

Fig. 3 Pemetrexed or cisplatin confers additive anti-proliferative effects in near-haploid mesothelioma after SETDB1 restoration. Additive effects were evaluated in near-haploid mesothelioma cell lines (MESO257 and MESO542) after lentiviral mediated *SETDB1* restoration and pemetrexed or cisplatin treatment in assays assessing cell viability (**a**), colony formation (**b**, **c**), xenograft growth (**d**, **e**) and immunoblotting (**f**). **a** Cell viability was evaluated by CellTiter Glo® ATP-based luminescence assay, 72 h after treatment with pemetrexed. Data were normalised to Lenti-GFP control and represent mean values (±s.d.) from quadruplicate cultures and were averaged from two independent experiments for each cell line. Statistically significant differences between Lenti-GFP control and SETDB1-GFP construct infections are presented as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. **b** Colony growth assays were performed after treatment with pemetrexed for 5 days. Colony growth experiments were performed in triplicate. Combination pemetrexed treatment and SETDB1 restoration led to a greater reduction in colony formation in MESO257 and MESO542 than either intervention alone. Scale bar: 3.5 mm. **c** Quantitation of cell colony growth in MESO257 and MESO542 with stably expressed SETDB1 constructs after treatment with pemetrexed. Statistically significant differences between Lenti-GFP + H_2O control and SETDB1-GFP + pemetrexed are presented as $**p < 0.01$ and $***p < 0.001$. **d** Combination of pemetrexed treatment and lentiviral-mediated SETDB1 restoration inhibited mesothelioma xenograft growth more effectively than either intervention alone. **e** Mesothelioma xenograft weights for mice with Lenti-GFP + H_2O controls and SETDB1 + pemetrexed. Statistically significant differences between Lenti-GFP + H_2O control and SETDB1-GFP+ pemetrexed are presented as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. **f** Immunoblotting evaluated expression of cyclin A in near-haploid mesothelioma (MESO542: Lenti-GFP and SETDB1-GFP restoration) xenografts after treatment with pemetrexed for 7 days. GAPDH stain is a loading control. **g** Cell viability was evaluated by a CellTiter Glo® ATP-based luminescence assay in near-haploid cell lines (MESO542 and MESO257) with stably expressed SETDB1-GFP restoration, 72 h after treatment with cisplatin (CIS, 2.5 and 5 μM) and/or pemetrexed (PEM, 0.25 μM). Data were normalised to Lenti-GFP control and represent mean values (±s.d.) from quadruplicate cultures, averaged from two independent experiments for each cell line. Statistically significant differences between Lenti-GFP control and SETDB1-GFP construct infections are presented as $****p < 0.0001$.

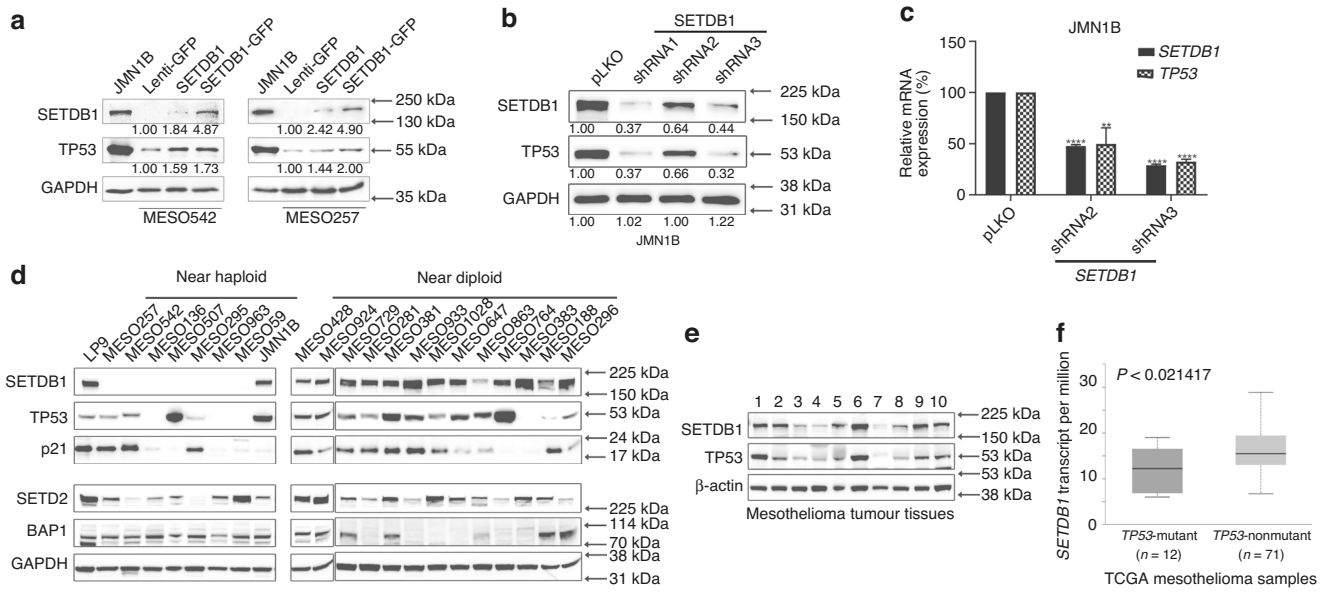

Fig. 4 SETDB1 is positively associated with TP53 expression. a Immunoblotting evaluation of TP53 and SETDB1 in near-haploid mesothelioma cell lines (MESO257 and MESO542) after lentiviral SETDB1 restoration. GAPDH stain is a loading control. Near-haploid mesothelioma cell line JMN1B is a positive control for SETDB1. **b** Immunoblotting evaluation of SETDB1 and TP53 expression in the near-haploid mesothelioma cell line JMN1B after lentiviral SETDB1 knockdown for 96 h. GAPDH stain is a loading control. **c** Quantitative RT-PCR evaluations of *SETDB1* and *TP53* transcripts at 96 h after infection of JMN1B cells with lentiviral *SETDB1* shRNA constructs. Data were normalised to pLKO control and represent the mean values (±s.d.) from triplicate assays, averaged from two independent experiments. Statistically significant differences between pLKO control and lentiviral shRNA treatment are presented as $^{**}p < 0.01$, $^{****}p < 0.0001$. **d** Immunoblotting evaluation of the expression of SETDB1, TP53, p21, SETD2, and BAP1 in normal mesothelial cell LP9, eight near-haploid mesothelioma cell lines and thirteen near-diploid mesothelioma cell lines. GAPDH staining is a loading control. **e** Immunoblotting evaluation of the expression of SETDB1 and TP53 in mesothelioma primary frozen tumours. Actin staining is a loading control. **f** TCGA gene expression profiling dataset analysis on 83 mesotheliomas shows that SETDB1 expression is stronger in *TP53*-nonmutant mesothelioma patient samples than in *TP53*-mutant mesothelioma.

The possibility of complexing between SETDB1 and TP53 was assessed by TP53 immunoprecipitation in near-diploid MESO924 cells which co-express SETDB1 and TP53 (Fig. S3). These studies did not demonstrate physical interaction between SETDB1 and TP53 (Fig. S3).

DISCUSSION

Available therapies, including surgery, radiation, chemotherapy, target therapies, and immunotherapies have not substantially improved survival for patients with mesothelioma. Hence, there is an urgent need to validate novel and biologically rational therapies for this invariably lethal disease. Recent studies have shown that SETDB1 is inactivated in ~3% of mesotheliomas overall [7], particularly in near-haploid cases [7], but the biologic roles of SETDB1 in mesothelioma are unknown [33, 34].

Our analyses of protein lysates from near-haploid vs. near-diploid mesothelioma cultures demonstrated that SETDB1 expression was lost in seven of eight near-haploid mesotheliomas but retained in each of 13 near-diploid mesotheliomas (Figs. 1a and 4d). Therefore, SETDB1 expression appears to be a useful biomarker for distinction of near-haploid mesothelioma from near-diploid mesothelioma. The nearly ubiquitous loss of SETDB1 expression in near-haploid mesotheliomas is consistent with a central tumour suppressor role in this unique mesothelioma subtype.

Pemetrexed and cisplatin are used as first-line treatment for mesothelioma [35]. Pemetrexed/cisplatin treatment inhibited cell proliferation, colony formation, and cyclin A expression in MESO542 and MESO257 and these effects were magnified by SETDB1 restoration. The additive effects after STEDB1 restoration and pemetrexed/cisplatin treatment included greater reduction in viability (Fig. 3a, g), colony formation (Fig. 3b, c), cyclin A expression (Fig. 3f) and xenograft growth than with either intervention alone (Fig. 3d, e). These results highlight SETDB1 as a tumour suppressor whose restoration could enhance pemetrexed or cisplatin clinical efficacy in mesothelioma. By contrast, we did not demonstrate inactivation of the SETD2 histone methyltransferase (previously implicated in a small subset of mesothelioma [11, 36–38]) in either near-haploid or near-diploid mesotheliomas in this series.

Our previous studies demonstrated that co-targeting of PI3K/mTOR, AXL, FAK, and MDM2 suppressed mesothelioma growth by dysregulating MDM2-TP53 interaction and/or inducing TP53 expression [4, 30, 31]. In the current study, we determined whether SETDB1 regulated mesothelioma TP53 expression. This work builds upon observations of SETDB1 and TP53 cross-regulation in other types of cancer. Specifically, SETDB1 impacts TP53 expression in liver cancer, likely because the SETDB1 histone methyltransferase function modulates TP53 methylation: SETDB1 knockdown also inhibited TP53 Ser15 phosphorylation in this setting [32]. Likewise, SETDB1 and TP53 demonstrate cross-regulation in lung cancers [39], and TP53 modulates SETDB1 expression in other settings [40]. We show herein that SETDB1 restoration in near-haploid mesothelioma up-regulates TP53 expression, in vitro and in vivo (Figs. 4a and 5). Further, SETDB1 silencing down-regulates TP53 protein and mRNA in JMN1B, which was the one near-haploid mesothelioma line retaining SETDB1 expression (Fig. 4b, c). SETDB1 and TP53 expression levels were also positively associated in mesothelioma clinical specimens (Fig. 4e). After SETDB1 restoration in near-haploid mesotheliomas (MESO542 and MESO257), *TP53* knockdown reduced SETDB1 transcript and protein expression and accordingly reduced di/tri-methyl H3K9 (Fig. 5a, b). These observations suggest that the observed co-inactivation of TP53 and SETDB1 in near-haploid mesotheliomas might dysregulate

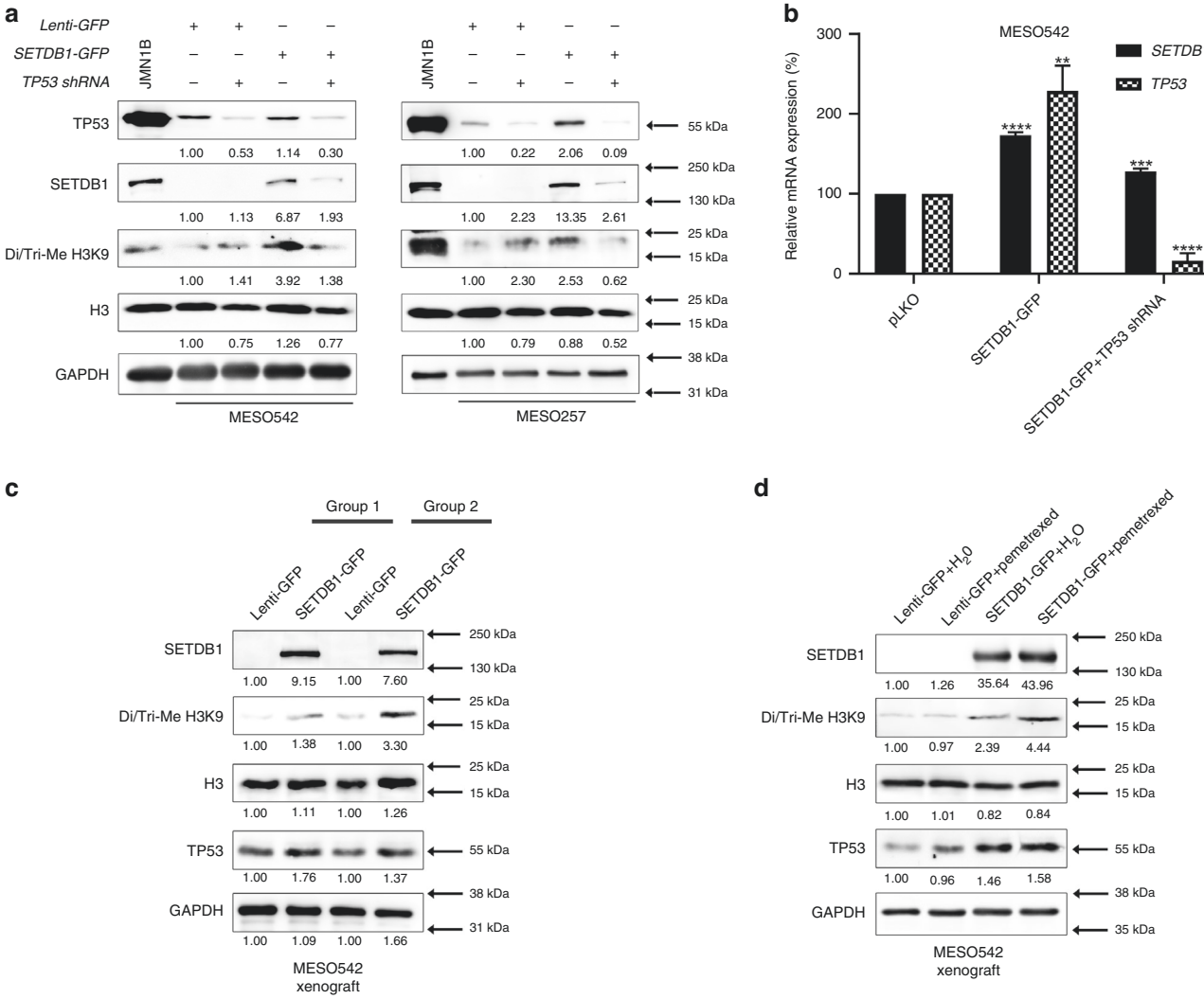

Fig. 5 The tumour suppressor roles of SETDB1 associated with TP53 expression were evaluated after TP53 knockdown in near-haploid mesothelioma cells and xenografts with SETDB1 restoration. a Immunoblotting evaluated expression of SETDB1, Di/Tri-methyl H3K9, and TP53 in MESO542 and MESO257 with stably expressed SETDB1 at 96 h post-infection with lentiviral *TP53* shRNA. GAPDH stain is a loading control. Near-haploid mesothelioma cell line JMN1B is positive control. **b** Quantitative RT-PCR evaluations of *SETDB1* and *TP53* transcripts at in MESO542 with stably expressed SETDB1 at 96 h post-infection with lentiviral *TP53* shRNA. Data were normalised to Lenti-GFP control and represent the mean values (±s.d.) from triplicate assays, averaged from two independent experiments. Statistically significant differences between Lenti-GFP control and lentiviral shRNA treatment are presented as $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. **c** Immunoblotting evaluation of expression of SETDB1, Di/Tri-methyl H3K9, H3, and TP53 in near-haploid mesothelioma (MESO542: Lenti-GFP and SETDB1-GFP restoration) xenografts. GAPDH stain is a loading control. **d** Immunoblotting evaluation of expression of SETDB1, Di/Tri-methyl H3K9, H3, and TP53 in near-haploid mesothelioma (MESO542: Lenti-GFP and SETDB1-GFP restoration) xenografts after treatment with pemetrexed for 7 days. GAPDH stain is a loading control.

oncogenic pathways jointly coordinated by these two transcriptional master switches.

In this study, four of eight near-haploid mesothelioma cell lines harboured *TP53* mutations, of which two resulted in loss of TP53 protein expression (MESO136 and MESO59) and two were missense mutations resulting in TP53 protein overexpression (MESO507 and JMN1B). Each of these manifested loss of p21 expression, consistent with compromised TP53 transactivating function (Fig. 4d and Table S2). An additional near-haploid case (MESO963) lacked TP53 expression and had loss of p21 expression but had no demonstrable *TP53* genomic mutation. Of the five near-haploid cases with aberrant TP53, four cases (all except JMN1B) had concurrent extinction of SETDB1 expression. These data corroborate co-inactivation of TP53 and SETDB1 in near-haploid mesothelioma, which is consistent with recent findings that concurrent mutation of *SETDB1* and *TP53* are associated with near-haploidisation in peritoneal mesothelioma (concurrent *TP53* and *SETDB1* mutations in 1 of 3 near-haploid cases) [36] and pleural mesothelioma (concurrent *TP53* and *SETDB1* mutations in 4 of 5 near-haploid cases) [7]. Based on genomic evidence, Hmeljak et al. hypothesised that *TP53* mutations resulted in mesothelioma near-haploidy, which in some cases was then followed by genome duplication and *SETDB1* inactivation [7]. The evidence for this model included apparent presence of heterozygous *SETDB1* mutations in 2 of 3 mesotheliomas which had undergone near-haploidisation. However, in the current study, seven of eight near-haploid mesotheliomas had complete loss of SETDB1 expression, in keeping with *SETDB1* inactivation during the near-haploid phase rather than the previously proposed haploinsufficient inactivation occurring after genome duplication. We also note that the previously published observation of apparent *SETDB1* mutation heterozygosity has alternate explanations, including

heterogeneity in the near-haploid population, with some cells containing *SETDB1* mutation and other cells not. Similarly, the hypothesis that TP53 mutation is an early event driving near-haploidisation begs the question as to how near-haploidisation also occurs in mesotheliomas lacking apparent TP53 inactivation, as was seen in three of eight cases in this series (MESO257, MESO542, and MESO295). Finally, we note that persistent near-haploid cells, with chromosome counts ranging from 24 to 28, were identified by karyotyping in 4 of the 8 near-haploid cases in this series (Table S1), even though these cells were from bulky, advanced, pleural mesotheliomas. This observation indicates that the near-haploid phase persists in some mesotheliomas, rather than representing a transitory phase that must acquire genome duplication to foster aggressive clinical behaviour.

Notably, expression of the BAP1 mesothelioma tumour suppressor [11, 37, 38, 41] was retained in each of the eight near-haploid mesothelioma cultures in this series, whereas BAP1 expression was lost in six of 13 near-diploid mesothelioma cultures (Fig. 4d). These findings underscore distinct biologic profiles in near-haploid mesothelioma (frequent inactivation of SETDB1 and TP53, intact BAP1, nearly genome-wide loss of heterozygosity) vs. near-diploid mesothelioma (frequent BAP1 inactivation) [7].

In conclusion, our studies demonstrate frequent SETDB1 inactivation in near-haploid mesothelioma but not in near-diploid mesothelioma, and demonstrate SETDB1 roles in TP53 regulation. We also show that SETDB1 restoration inhibits near-haploid mesothelioma growth, and these effects are enhanced by co-treatment with pemetrexed and cisplatin, in vitro and in vivo. Our studies show that near-haploid mesothelioma differ, overall, from near-diploid mesothelioma not only due to a higher frequency of SETDB1 and TP53 inactivation but also due to low frequency of BAP1 inactivation. These findings define strategies for biomarker and therapeutics exploration in future studies.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article and its Supplementary Information files.

REFERENCES

1. Baumann F, Ambrosi J-P, Carbone M. Asbestos is not just asbestos: an unrecognised health hazard. Lancet Oncol. 2013;14:576–8.
2. Craighead J. Current pathogenetic concepts of diffuse malignant mesothelioma. Hum Pathol. 1987;18:544–57.
3. Järvholm B, Burdorf A. Emerging evidence that the ban on asbestos use is reducing the occurrence of pleural mesothelioma in Sweden. Scand J Public Health. 2015;43:875–81.
4. Song W, Wang H, Lu M, Ni X, Bahri N, Zhu S, et al. AXL inactivation inhibits mesothelioma growth and migration via regulation of p53 expression. Cancers. 2020;12:2757.
5. Sunil SK, Prakash PN, Hariharan S, Vinod G, Preethi RT, Geetha N, et al. Adult acute lymphoblastic leukemia with near haploidy, hyperdiploidy and Ph positive lines: a rare entity with poor prognosis. Leuk Lymphoma. 2006;47:561–3.
6. Neragi-Miandoab S, Sugarbaker DJ. Chromosomal deletion in patients with malignant pleural mesothelioma. Interact Cardiovasc Thorac Surg. 2009;9:42–4.
7. Hmeljak J, Sanchez-Vega F, Hoadley KA, Shih J, Stewart C, Heiman D, et al. Integrative molecular characterization of malignant pleural mesothelioma. Cancer Discov. 2018;8:1548–65.
8. Sukov WR, Ketterling RP, Wei S, Monaghan K, Blunden P, Mazzara P, et al. Nearly identical near-haploid karyotype in a peritoneal mesothelioma and a retroperitoneal malignant peripheral nerve sheath tumor. Cancer Genet Cytogenet. 2010;202:123–8.
9. Betti M, Aspesi A, Sculco M, Matullo G, Magnani C, Dianzani I. Genetic predisposition for malignant mesothelioma: a concise review. Mutat Res Rev Mutat Res. 2019;781:1–10.
10. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat Genet. 2011;43:668–72.
11. Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet. 2016;48:407–16.
12. Guo G, Chmielecki J, Goparaju C, Heguy A, Dolgalev I, Carbone M, et al. Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesothelioma. Cancer Res. 2015;75:264.
13. Kang HC, Kim HK, Lee S, Mendez P, Kim JW, Woodard G, et al. Whole exome and targeted deep sequencing identify genome-wide allelic loss and frequent SETDB1mutations in malignant pleural mesotheliomas. Oncotarget. 2016;7:8321–31.
14. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. 2002;16:919–32.
15. Matsui T, Leung D, Miyashita H, Maksakova IA, Miyachi H, Kimura H, et al. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature. 2010;464:927–31.
16. Minkovsky A, Sahakyan A, Rankin-Gee E, Bonora G, Patel S, Plath K. The Mbd1 - Atf7ip - Setdb1 pathway contributes to the maintenance of X chromosome inactivation. Epigenet Chromatin. 2014;7:12.
17. Song YJ, Choi JH, Lee H. Setdb1 is required for myogenic differentiation of C2C12 myoblast cells via maintenance of MyoD expression. Mol Cell. 2015;38:362–72.
18. Lawson KA, Teteak CJ, Gao J, Li N, Hacquebord J, Ghatan A, et al. ESET histone methyltransferase regulates osteoblastic differentiation of mesenchymal stem cells during postnatal bone development. FEBS Lett. 2013;587:3961–7.
19. Yang L, Lawson KA, Teteak CJ, Zou J, Hacquebord J, Patterson D, et al. ESET histone methyltransferase is essential to hypertrophic differentiation of growth plate chondrocytes and formation of epiphyseal plates. Dev Biol. 2013;380:99–110.
20. Regina C, Compagnone M, Peschiaroli A, Lena A, Annicchiarico-Petruzzelli M, Piro MC, et al. Setdb1, a novel interactor of ΔNp63, is involved in breast tumorigenesis. Oncotarget. 2016;7:28836–48.
21. Lafuente-Sanchis A, Zúñiga Á, Galbis JM, Cremades A, Estors M, Martínez-Hernández NJ, et al. Prognostic value of ERCC1, RRM1, BRCA1 and SETDB1 in early stage of non-small cell lung cancer. Clin Transl Oncol. 2015;18:798–804.
22. Sun Y, Wei M, Ren SC, Chen R, Xu WD, Wang FB, et al. Histone methyltransferase SETDB1 is required for prostate cancer cell proliferation, migration and invasion. Asian J Androl. 2014;16:319–24.
23. Ou WB, Corson JM, Flynn DL, Lu WP, Wise SC, Bueno R, et al. AXL regulates mesothelioma proliferation and invasiveness. Oncogene. 2011;30:1643–52.
24. Ou WB, Hubert C, Fletcher JA, Bueno R, Flynn DL, Sugarbaker DJ, et al. Targeted inhibition of multiple receptor tyrosine kinases in mesothelioma. Neoplasia. 2011;13:12–22.
25. Demetri GD, Zenzie BW, Rheinwald JG, Griffin JD. Expression of colony-stimulating factor genes by normal human mesothelial cells and human malignant mesothelioma cells lines in vitro. Blood. 1989;74:940–6.
26. Behbehani AM, Hunter WJ, Chapman AL, Lin F. Studies of a human mesothelioma. Hum Pathol. 1982;13:862–6.
27. Gordon GJ, Rockwell GN, Jensen RV, Rheinwald JG, Glickman JN, Aronson JP, et al. Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. Am J Pathol. 2005;166:1827–40.
28. Ou WB, Ni N, Zuo R, Zhuang W, Zhu M, Kyriazoglou A, et al. Cyclin D1 is a mediator of gastrointestinal stromal tumor KIT-independence. Oncogene. 2019;38:6615–29.
29. Chen WC, Kuang Y, Qiu HB, Cao Z, Tu Y, Sheng Q, et al. Dual targeting of insulin receptor and KIT in imatinib-resistant gastrointestinal stromal tumors. Cancer Res. 2017;77:5107–17.
30. Ou WB, Lu M, Eilers G, Li H, Ding J, Meng X, et al. Co-targeting of FAK and MDM2 triggers additive anti-proliferative effects in mesothelioma via a coordinated reactivation of p53. Br J Cancer. 2016;115:1253.
31. Zhou S, Liu L, Li H, Eilers G, Kuang Y, Shi S, et al. Multipoint targeting of the PI3K/mTOR pathway in mesothelioma. Br J Cancer. 2014;110:2479–88.
32. Fei Q, Shang K, Zhang J, Chuai S, Kong D, Zhou T, et al. Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. Nat Commun. 2015;6:8651.
33. Yoshikawa Y, Sato A, Tsujimura T, Otsuki T, Fukuoka K, Hasegawa S, et al. Biallelic germline and somatic mutations in malignant mesothelioma: multiple mutations in transcription regulators including mSWI/SNF genes. Int J Cancer. 2015;136:560–71.
34. Yuan L, Sun B, Xu L, Chen L, Ou W. The updating of biological functions of methyltransferase SETDB1 and its relevance in lung cancer and mesothelioma. Int J Mol Sci. 2021;22:7416.

35. Marcq E, Audenaerde JRV, Waele J, Jacobs J, Loenhout JV, Cavents G, et al. Building a bridge between chemotherapy and immunotherapy in malignant pleural mesothelioma: investigating the effect of chemotherapy on immune checkpoint expression. Int J Mol Sci. 2019;20:4182.
36. Offin M, Yang SR, Egger J, Jayakumaran G, Spencer RS, Lopardo J, et al. Molecular characterization of peritoneal mesotheliomas. J Thorac Oncol. 2022;17:455–60.
37. Carbone M, Adusumilli PS, Alexander HR Jr, Baas P, Bardelli F, Bononi A, et al. Mesothelioma: scientific clues for prevention, diagnosis, and therapy. CA Cancer J Clin. 2019;69:402–29.
38. Yoshikawa Y, Emi M, Hashimoto-Tamaoki T, Ohmuraya M, Sato A, Tsujimura T, et al. High-density array-CGH with targeted NGS unmask multiple noncontiguous minute deletions on chromosome 3p21 in mesothelioma. Proc Natl Acad Sci USA. 2016;113:13432–7.
39. Sun QY, Ding LW, Xiao JF, Chien W, Lim SL, Hattori N, et al. SETDB1 accelerates tumorigenesis by regulating WNT signaling pathway. J Pathol. 2015;235:559–70.
40. Noh HJ, Kim KA, Kim KC. p53 down-regulates SETDB1 gene expression during paclitaxel induced-cell death. Biochem Biophys Res Commun. 2014;446:43–8.
41. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet. 2011;43:1022–5.

ACKNOWLEDGEMENTS

We thank Zhejiang Provincial Key Laboratory of Silkworm Bioreactor and Biomedicine in Zhejiang Sci-Tech University for providing the experimental platform.

AUTHOR CONTRIBUTIONS

WBO and JAF designed the study; MX, YT, WB, MZL, and W-BO performed the experiments and acquired the data. MX, YT, WB, MZL, JAF, and W-BO analysed and interpreted the acquired data. YT, IK, JAF, and WBO participated in scientific discussion and drafting of the manuscript.

FUNDING

This research was supported by National Natural Science Foundation of China (82272695), the Key Program of Natural Science Foundation of Zhejiang Province (LZ23H160004), China. This work was also supported by the NIH/NCI SPORE 1P50CA272170-01 (JAF).

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Brigham and Women's Hospital and Zhejiang Sci-Tech University Institutional Ethics Boards approved this study. Patients provided informed consent for use of their tissue samples for research purposes. The study was performed in accordance with the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Not applicable.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41416-023-02330-x.

Correspondence and requests for materials should be addressed to Wen-Bin Ou.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.