Human Molecular Genetics, 2019, Vol. 28, No. 17 2826–2834

doi: 10.1093/hmg/ddz112
Advance Access Publication Date: 17 April 2019
General Article

GENERAL ARTICLE

PTEN modulates gene transcription by redistributing genome-wide RNA polymerase II occupancy

Ata Abbas[1,†], Roshan Padmanabhan[1], Todd Romigh[1] and Charis Eng[1,2,3,4,*]

[1]Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA, [2]Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH 44195, USA, [3]Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, OH 44116, USA and [4]Germline High Risk Focus Group, Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH 44116, USA

*To whom correspondence should be addressed at: Tel: (216) 444-3440; Fax: (216) 636-0655; Email: engc@ccf.org

Abstract

Control of gene expression is one of the most complex yet continuous physiological processes impacting cellular homeostasis. RNA polymerase II (Pol II) transcription is tightly regulated at promoter-proximal regions by intricate dynamic processes including Pol II pausing, release into elongation and premature termination. Pol II pausing is a phenomenon where Pol II complex pauses within 30–60 nucleotides after initiating the transcription. Negative elongation factor (NELF) and DRB sensitivity inducing factor (DSIF) contribute in the establishment of Pol II pausing, and positive transcription elongation factor b releases (P-TEFb) paused complex after phosphorylating DSIF that leads to dissociation of NELF. Pol II pausing is observed in most expressed genes across the metazoan. The precise role of Pol II pausing is not well understood; however, it's required for integration of signals for gene regulation. In the present study, we investigated the role of phosphatase and tensin homolog (*PTEN*) in genome-wide transcriptional regulation using PTEN overexpression and PTEN knock-down models. Here we identify that PTEN alters the expression of hundreds of genes, and its restoration establishes genome-wide Pol II promoter-proximal pausing in PTEN null cells. Furthermore, PTEN re-distributes Pol II occupancy across the genome and possibly impacts Pol II pause duration, release and elongation rate in order to enable precise gene regulation at the genome-wide scale. Our observations demonstrate an imperative role of PTEN in global transcriptional regulation that will provide a new direction to understand PTEN-associated pathologies and its management.

Introduction

Phosphatase and tensin homolog (*PTEN*) is a well-studied tumor suppressor gene on 10q23.3 frequently mutated or deleted in diverse sporadic cancers and the autosomal dominant Cowden syndrome (OMIM 158350) characterized by a high risk of breast, thyroid and other cancers (1–7). PTEN is a dual specificity phosphatase that dephosphorylates both lipids and proteins (8). It canonically antagonizes PI3K-AKT-mTOR pathway due to its phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase activity, thereby regulating cell growth, proliferation and survival (9,10). PTEN expresses ubiquitously and undergoes extensive post-translational modifications that regulate PTEN protein stability, activity, protein–protein interaction and localization (9,11,12).

†Present address: Division of Hematology and Oncology, Department of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA.
Received: March 5, 2019. **Revised:** April 27, 2019. **Accepted:** May 20, 2019

Formerly believed only to be cytoplasmic, PTEN also localizes to the nucleus and exhibits tumor suppressor functions, possibly through both its phosphatase and non-phosphatase activity (13,14). In the nucleus, PTEN helps in maintenance of genomic integrity, homologous recombination and repair of DNA double-strand breaks (15–17). It also regulates cellular senescence and cell cycle progression (18–20). PTEN interacts with a wide variety of nuclear factors including p53, CENP-C, HIF1α, CREB and MCRS1 (21–23). Intriguingly, PTEN contribution in regulating chromatin condensation suggests its broader role in transcriptional regulation (24,25). A study by Horita *et al*. (26) reported the interaction of serum response factor with PTEN that regulates smooth muscle-specific genes. rRNA transcription is also regulated by PTEN and its alternatively translated isoform PTENβ (27). Numerous genome-wide transcription studies, including both in human cell line and animal models, strongly suggest the involvement of PTEN in the (direct and indirect) regulation of hundreds of genes (28,29). However, the precise mechanism of PTEN in regulating global transcription is not known.

Gene transcription is a complex process and tightly regulated at multiple levels. Rpb1, the largest subunit of RNA Polymerase II (Pol II) complex, contains an unusual C-terminal domain (CTD) comprising the highly conserved 25–52 tandemly repeated heptad sequence YSPTSPS. The dynamic post-translational modifications of Pol II CTD provide an interactive platform for various factors required for transcription initiation, elongation, termination, as well as RNA processing (30). Once transcription starts, Pol II pauses within 30–60 nucleotides from the transcription start site (TSS) (31). The precise role of Pol II pausing is not well known. Pol II pausing can be retained or acquired during transcriptional responses (32), suggesting a possible decision-making mechanism taking place at the paused site. As such, we hypothesized that nuclear PTEN contributes to the regulation of RNA Pol II pausing dynamics to maintain transcriptional homeostasis. Considering the known functions of nuclear PTEN and its probable involvement in gene transcription, we, therefore, sought to understand the in-depth role of PTEN in global transcriptional regulation.

Results

PTEN modulates global gene expression

To understand how PTEN contributes to genome-wide transcriptional regulation, we have generated a stable PTEN wild-type expressing line using the BT-549 (PTEN null) cells. In this overexpression model, a robust expression of exogenous 3X FLAG-tagged PTEN was observed and functionally active as evidenced by downregulation of pAKT (Fig. 1A; Supplementary Material, Fig. S1A). RNA sequencing (RNA-seq) analysis revealed that wild-type PTEN overexpression in BT-549 cells resulted in 2146 differentially expressed genes (DEGs) (FDR-corrected *P*-value <0.05) (Fig. 1B; Supplementary Material, Fig. S1B). We confirmed our RNA-seq results by examining the expression of few selected genes using reverse transcription (RT-qPCR) method (Supplementary Material, Fig. S1C). We performed pathway analysis using Ingenuity Pathway Analysis (IPA, Qiagen Bioinformatics, Redwood City, CA), which revealed these DEGs clustering in diverse canonical signaling pathways including BRCA1 in DNA damage, mismatch repair, AMPK signaling, EIF2 signaling, iNOS signaling, NFκB signaling, etc. (Fig. 1C).

In another model using MCF7 cells, we knock down PTEN using short hairpin RNA (shRNA) (Fig. 1D) and performed RNA-seq analysis. PTEN knock-down in MCF7 cells resulted in 1242 DEGs (FDR-corrected *P*-value <0.05) (Fig. 1E; Supplementary Material, Fig. S1D). Gene ontology (GO) analysis using IPA showed various canonical signaling pathways including protein ubiquitination, AHR signaling, DNA methylation and transcriptional repression signaling and p70S6K signaling (Fig. 1F). Remarkably, we found that 137 genes that were relatively overexpressed due to wild-type PTEN overexpression in BT-549 cells were also relatively underexpressed due to PTEN knock-down in MCF7 cells. Furthermore, 134 genes that were underexpressed in BT-549_PTEN cells were found overexpressed in MCF7_PTEN-shRNA cells (Supplementary Material, Fig. S1E).

PTEN expression regulates genome-wide RNA Pol II pausing

To get an in-depth understanding of transcriptional processes and its modulation by PTEN, we performed RNA Pol II chromatin immuno-precipitation sequencing (ChIP-seq) analysis. We observed a significant global increase in Pol II pausing at promoter proximal region after PTEN overexpression in BT-549 cells (Fig. 2A and B; Supplementary Material, Fig. S2A). However, a small group of genes (n = 136) had decreased Pol II pausing after PTEN re-expression in BT-549 cells (Fig. 2C and D; Supplementary Material, Fig. S2B). After reducing PTEN expression by adding tetracycline in BT-549-PTEN cells (pTet-Off expression system) for 48 h, we observed a decrease in Pol II pausing (Fig. 2E) confirming an intriguing role of PTEN in the maintenance of Pol II pausing. Furthermore, to confirm the role of PTEN in maintaining Pol II pausing, we knocked down PTEN using shRNA and performed Pol II ChIP-seq analysis. We observed a decrease in Pol II pausing at promoter-proximal regions, further confirming a new role of PTEN in the maintenance of Pol II pausing (Fig. 2F and G; Supplementary Material, Fig. S2C). Interestingly, only a small group of genes (n = 89) showed a modest increase in Pol II occupancy at promoter proximal regions after PTEN knock-down in MCF7 cells (Supplementary Material, Fig. S2D and E).

PTEN modulates RNA Pol II pause release

RNA Pol II complex enters into productive elongation phase after release from pausing. Regulation of pause release into elongation contributes in overall transcriptional outcome. We calculated Pol II pause release ratio (PRR) by taking the ratio of gene body (+300 bp to +2 kb from TSS) counts to promoter (−50 bp to +300 bp form TSS) counts (see Materials and Methods for details). As expected, we observed an overall decrease in PRR after PTEN re-expression in BT-549 cells (10 141 genes, $P < 0.0001$) (Fig. 3A). Furthermore, we categorized these genes and noted that over 60% genes (n = 6269) exhibit decreased PRR after PTEN re-expression, while PRR of 2403 genes remained the same (Fig. 3B). Surprisingly, a significant increase in PRR was observed in 1479 genes ($P < 0.0001$) (Fig. 3B; Supplementary Material, Fig. S3A). GO analysis revealed that they are mainly involved in transcriptional regulation (Fig. 3C; Supplementary Material, Fig. S3B). To re-confirm the role of PTEN in Pol II pause release, we calculated the PRR of 14 300 genes in MCF7 cells after PTEN knock-down. We observed an overall increase in PRR ($P < 0.0001$) corroborating our previous findings (Fig. 3D). Among them, 5686 genes showed increased PRR ($P < 0.0001$), 7483 genes had no change, while 2231 genes

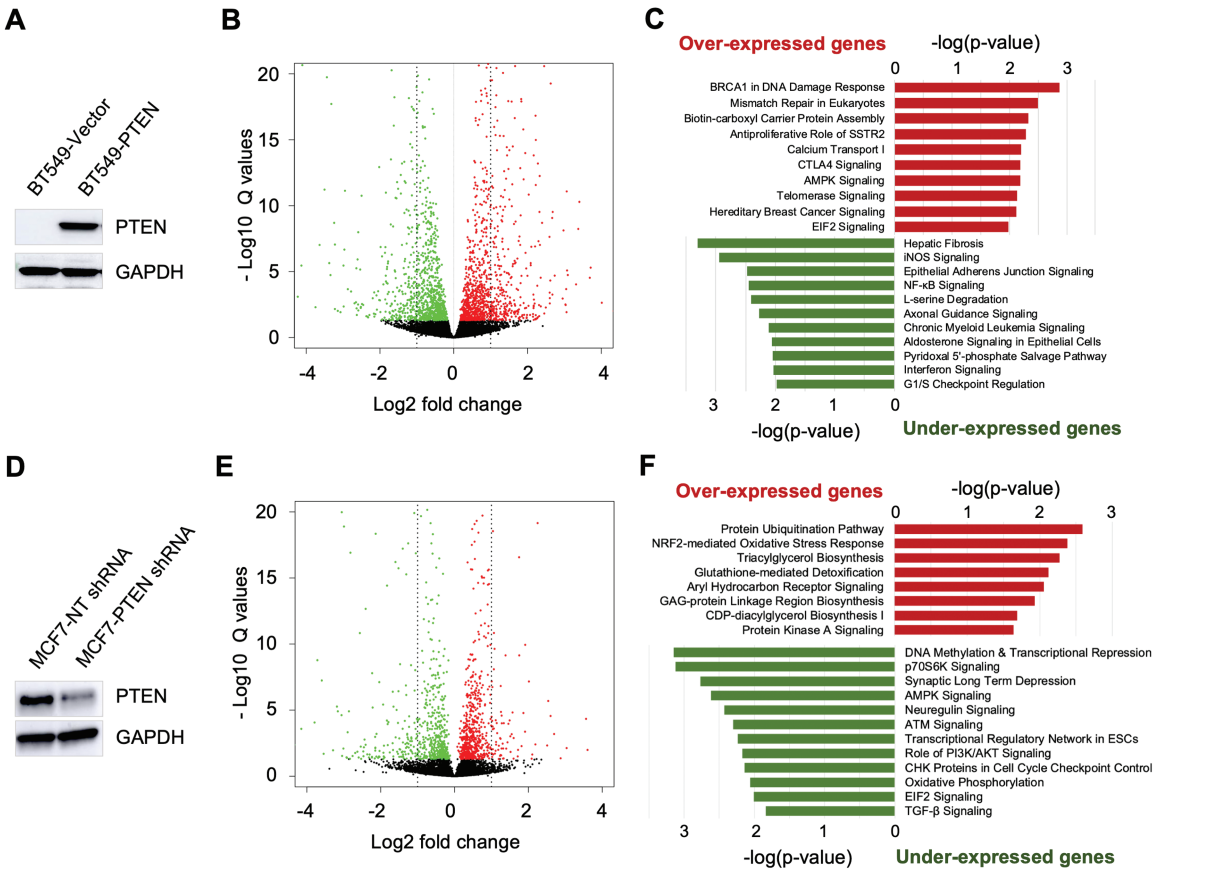

Figure 1. PTEN modulates global gene expression. **(A)** Overexpression of PTEN in BT-549 cells. **(B)** Volcano plot showing differential gene expression in BT-549 cells after overexpression of PTEN followed by RNA-seq analysis. A total of 2146 genes were differentially expressed (FDR-corrected *P*-value <0.05) including 400 and 299 genes 2-fold or more overexpressed and underexpressed, respectively. **(C)** IPA analysis of top canonical pathways in BT-549 cells with and without PTEN overexpression. **(D)** Immunoblot showing knock-down of PTEN in MCF7 cells. **(E)** Volcano plot showing differential gene expression (1242 genes, FDR-corrected *P*-value <0.05) in MCF7 cells after knock-down of PTEN using shRNA followed by RNA-seq analysis. With ≥2-fold change cut-off, 167 genes are relatively underexpressed and 87 genes are overexpressed. **(F)** IPA analysis of top canonical pathways of DEGs in MCF7 cells followed by PTEN knock-down.

exhibited a decrease in PRR after PTEN knock-down in MCF7 cells ($P < 0.0001$, Fig. 3E; Supplementary Material, Fig. S3C). The top GO categories of genes with decreased PRR ($n = 2231$) were inflammatory responses and cell migration (Fig. 3F; Supplementary Material, Fig. S3D).

Since PTEN null BT-549 cells have exhibited diminished Pol II occupancy at promoter proximal region in most of the genes, we hypothesized that lack of PTEN may be causing very rapid Pol II turnover in BT-549 cells. To test this hypothesis, we used TFIIH inhibitor Triptolide (Trp) to block transcription initiation followed by Pol II ChIP-qPCR analysis using specific primers targeting promoter proximal regions (see Supplementary Material for primer list). BT-549-Vector and BT-549-PTEN cells were treated with Trp (0.5 mM) for 1 and 2 min, and Pol II ChIP signals (% input) were used to generate a decay curve. Our Trp experiments demonstrated a rapid turnover of paused Pol II in PTEN null BT-549 cells compared to PTEN re-expressing BT-549 cells (Fig. 3G). This was an intriguing observation suggesting a possible role of PTEN in modulating Pol II pause duration or pause release. We have illustrated this with three genes (*FOS*, *SOX4* and *SRSF3*) using ChIP-qPCR. Our observations here would suggest a future genome-wide study, using more precise techniques such as PRO-seq, etc. that will be needed to understand the impact of PTEN on Pol II pause duration and release.

PTEN redistributes genome-wide Pol II occupancy

We observed a positive correlation between PTEN levels and Pol II occupancy at various locations across the genome including enhancers and regions encoding micro RNA (miRNA) and long interspersed non-coding RNA (Supplementary Material, Fig. S4A–C). Furthermore, PTEN overexpression in BT-549 cells led to re-distribution and increased Pol II occupancy across the gene body (Fig. 4A). Surprisingly, Pol II occupancy was diminished across the genome in PTEN null BT-549 cells possibly due to rapid Pol II turnover. To rule out any possible artifact, we compared our data with publicly available Pol II ChIP-seq datasets of different cell types. We re-analyzed Pol II ChIP-seq datasets of various cells including breast epithelium, T47D, MCF7, MDA-MB-231, iPSCs and dermal fibroblasts. We observed that the patterns of Pol II pausing and overall occupancy across the gene body were highly conserved across various cell types (Fig. 4B). Furthermore, knock-down of PTEN expression in MCF7 cells resulted in the converse, thus corroborating the observation in BT-549-PTEN overexpression model (Fig. 4C).

Knowing the direct involvement of PTEN in RNA splicing (33) and PTEN-dependent increases in Pol II occupancy on the gene body, we postulated that PTEN might play some role in regulating Pol II elongation rate, hence impacting co-transcriptional events. PTEN null and PTEN re-expressing BT-549 cells were treated with Trp (block transcription initiation

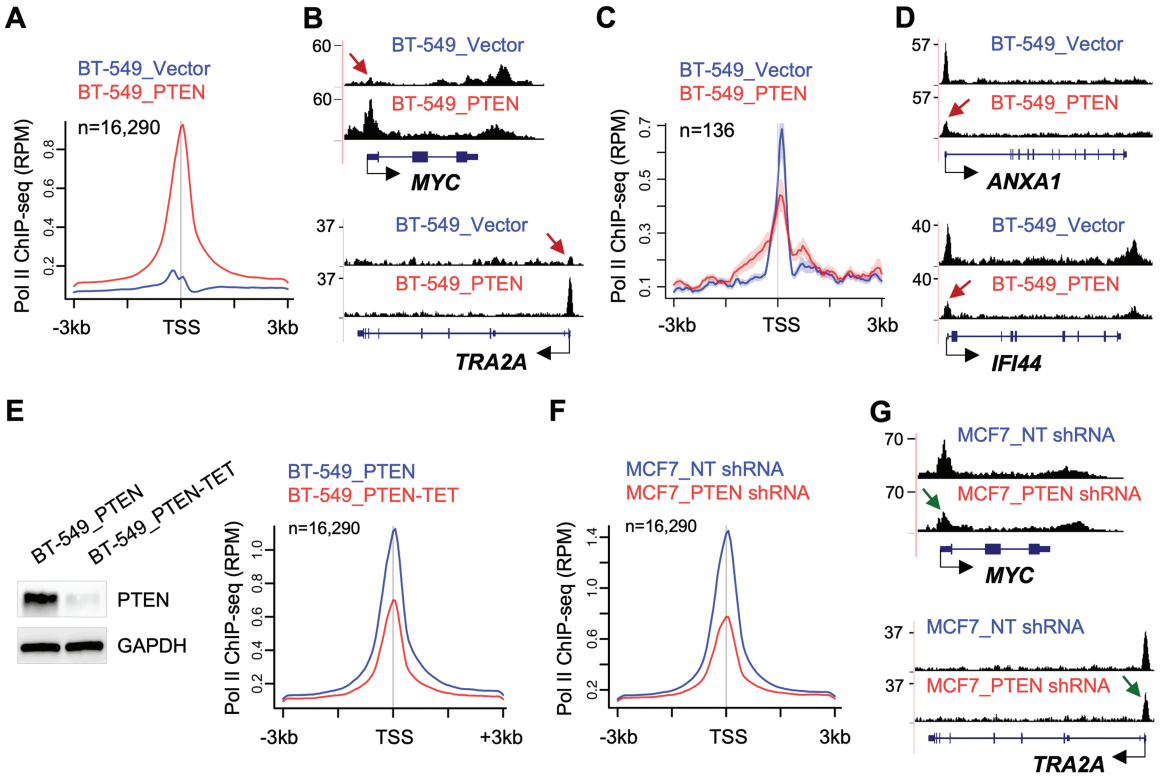

Figure 2. PTEN expression regulates genome-wide RNA Pol II pausing. **(A)** Metagene plot showing Pol II signals at TSS (average of 16 290 genes) in BT-549 cells after re-expression of PTEN followed by Pol II ChIP-seq. **(B)** Pol II occupancy at representative genes are shown using UCSC browser. **(C)** A small group of genes ($n = 136$) showing decrease in Pol II pausing after PTEN overexpression. **(D)** Decrease in Pol II occupancy after PTEN re-expression in BT-549 cells at representative genes are shown using UCSC browser. **(E)** PTEN expression after 48 h of tetracycline treatment (left), and Metagene plot showing Pol II signals at TSS in BT-549-PTEN +/− tetracycline treatment followed by Pol II ChIP-seq (right). **(F)** Metagene plot showing Pol II signals at TSS (average of 16 290 genes) in MCF7 cells after knock-down of PTEN using shRNA followed by Pol II ChIP-seq. **(G)** Pol II occupancy at representative genes are shown using UCSC browser.

by inhibiting TFIIH) for 5 and 10 min before Pol II occupancy at the terminal exon of representative genes were measured by ChIP-qPCR. Genes were selected based on their size (~10–25 kb) so that elongating Pol II complex reaching the terminal exon after 5 and 10 min of Trp treatments could be measured. Intriguingly, we observed increased Pol II levels at the last exons of the genes in BT-549-PTEN cells, suggesting a relatively slow elongation rate in PTEN expressing cells (Fig. 4D–F; Supplementary Material, Fig. S5A and B). Interestingly, no significant difference in elongation rate was observed in *IFI44* gene that was already paused in PTEN null cells and exhibited reduced Pol II pausing due to PTEN overexpression; further support the role of PTEN in modulating Pol II elongation rate (Supplementary Material, Fig. S5C).

Discussion

Transcriptional dysregulation is a hallmark of almost all types of cancers. Due to rapid emergence of cancer drug resistance, transcriptional regulators are promising therapeutic targets for a broad range of cancer therapies (34,35). The knowledge regarding the effects of available therapies on transcriptional control in patients is lacking. Since cancer development, progression and its phenotypes are defined by alterations in specific transcriptional networks (e.g. MYC-regulated cancers), in-depth understanding and mechanistic insight of transcriptional regulation are necessary to develop transcriptional inhibitor-based novel therapies in the future. Our current observations along with other published transcriptome studies (28,29,33) strongly suggest that PTEN plays a much wider role in the regulation of genome-wide transcription, thus strengthening its far-reaching implications from molecular diagnostics to therapeutics.

Pol II pausing is a stable, highly conserved, yet dynamic regulatory process during transcription. Pol II pausing is prevalent across the metazoan genomes and can regulate the expression of pivotal genes that code for transcription factors and signaling proteins (36,37). Pol II competes with nucleosomes for promoter-proximal occupancy in order to prevent formation of repressive chromatin (38). Henriques *et al.* (39) suggested that Pol II pausing provides a platform for the integration of regulatory signals. A recent report revealed that tumor suppressor BRCA2 regulates Pol II pause release to prevent R-loop formation and DNA damage (40). CTR9, another tumor suppressor, is a component of PAF1 complex that regulates promoter proximal Pol II pausing (41). Here we present the first direct observation of PTEN maintaining Pol II promoter-proximal pausing in two different cell lines (Fig. 2). Our data build on the above published works and point to an integral role for Pol II pausing in global transcriptional regulation.

Recent studies suggest that Pol II undergoes rapid turnover after pausing (42,43). Our data indicate that PTEN may play a critical role in regulating Pol II pause release and/or pause duration at the promoter-proximal region (Fig. 3). Since genome-wide Pol II pausing was increased due to PTEN re-expression in BT-549 cells, overall decrease in PRR was expected. We identified a small subgroup that exhibited significant increase in PRR

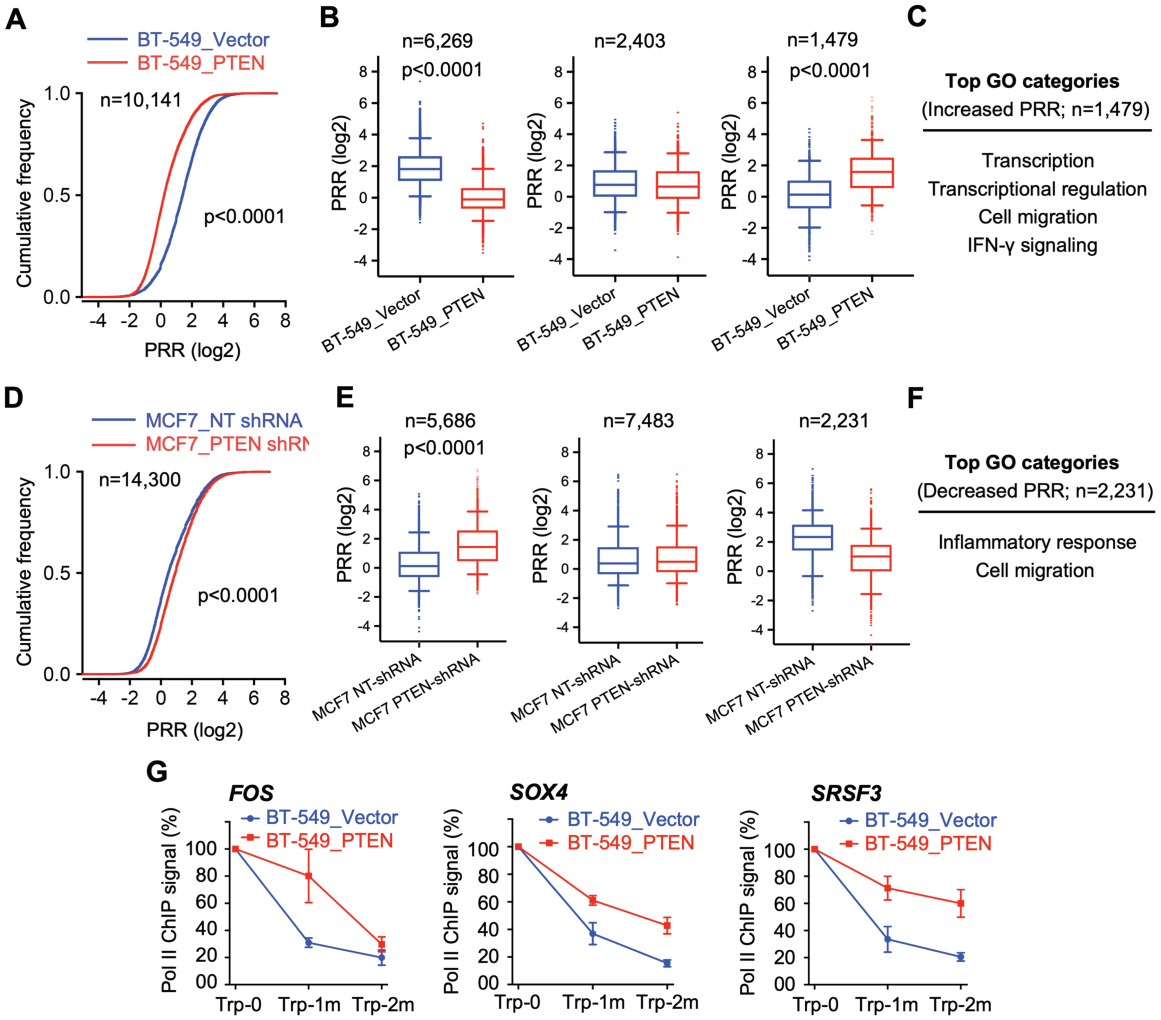

Figure 3. PTEN regulates RNA Pol II pause release. **(A)** PRR was calculated by taking the ratio of gene body (+300 bp to +2 kb from TSS) counts to promoter (−50 bp to +300 bp form TSS) counts. PRR is represented by cumulative frequency plots showing an overall decrease in PRR after re-expression of PTEN in BT-549 PTEN-null cells. **(B)** All the genes (n = 10.141) in panel 'A' were classified in to three groups based on their PRR. Genes are categorized under groups representing decreased (left, ≥1.5-fold reduction in PRR after PTEN re-expression in BT-549 cells), unchanged (center, between 0.67 and 1.5-fold change in PRR) and increased (right, ≥1.5-fold increase) PRR after PTEN expression in BT-549 cells. **(C)** Top GO categories for genes with an increase in PRR after PTEN re-expression in BT-549 cells. **(D)** Cumulative frequency plot representing an overall increase in PRR after knock-down of PTEN in MCF7 cells (n = 14 300 genes). **(E)** Box plots represent changes in PRR in three groups of genes. Genes are grouped based on their PRR: increased PRR group (left), ≥1.5-fold increase in PRR after PTEN knock-down in MCF7 cells; unchanged PRR group (center), between 0.67 and 1.5-fold change in PRR; and decreased PRR group (right), ≥1.5-fold reduction in PRR after PTEN knock-down in MCF7 cells. **(F)** Top GO categories for genes with a decrease in PRR after knock-down of PTEN in MCF7 cells. **(F)** BT-549 cells were treated with 0.5 mM Trp for 1 and 2 min and Pol II ChIP signals (% input) were used to generate a decay curve. Representative genes showing increase in Pol II pause duration after re-expression of PTEN in BT-549 cells.

mostly enriched with genes involved in the transcriptional regulation (Fig. 3B; Supplementary Material, Fig. S3A and B). This very intriguing observation further suggests a strong role of PTEN in transcriptional regulation. Furthermore, we elucidated that *FOS*, *SOX4* and *SRSF3* genes undergo rapid turnover of paused Pol II in PTEN-deficient BT-549 cells compared to PTEN re-expressing BT-549 cells (Fig. 3G). However, we do not know precisely how PTEN stabilizes the Pol II pausing at a genome-wide scale nor how it regulates Pol II pause release. We postulate that PTEN may impact recruitment of positive transcription elongation factor b (P-TEFb) and super elongation complex (SEC) in order to regulate Pol II pausing and pause release. Future investigations will need to evaluate the possible involvement of PTEN in regulating P-TEFb and/or SEC to get a more in-depth understanding of Pol II turnover at promoter-proximal regions. Understanding the exact mechanism will help in identifying newer therapeutic targets for PTEN-related pathologies.

RNA splicing is regulated by co-transcriptional events and is often regulated by Pol II elongation rate (44). Cells maintain a diverse RNA and proteins populations by using alternative splicing. Changes in splicing patterns may result in tumorigenesis (45). Very recently, Shen *et al.* (33) demonstrated that PTEN regulates several hundreds of alternative splicing events that are associated with the worst outcomes for cancer patients. Our data demonstrating the involvement of PTEN in modulating Pol II elongation rate (Fig. 4D–F; Supplementary Material, Fig. S5) begin to provide functional insight into these somatic clinical observations and provide further evidence that PTEN modulates alternative splicing, likely via co-transcription.

In conclusion, PTEN, encoded by a tumor suppressor cancer predisposition gene, plays a role in the maintenance of Pol II pausing, and possibly modulates Pol II elongation rate, suggesting a much deeper non-canonical function than previously suspected. However, further work is needed to understand its

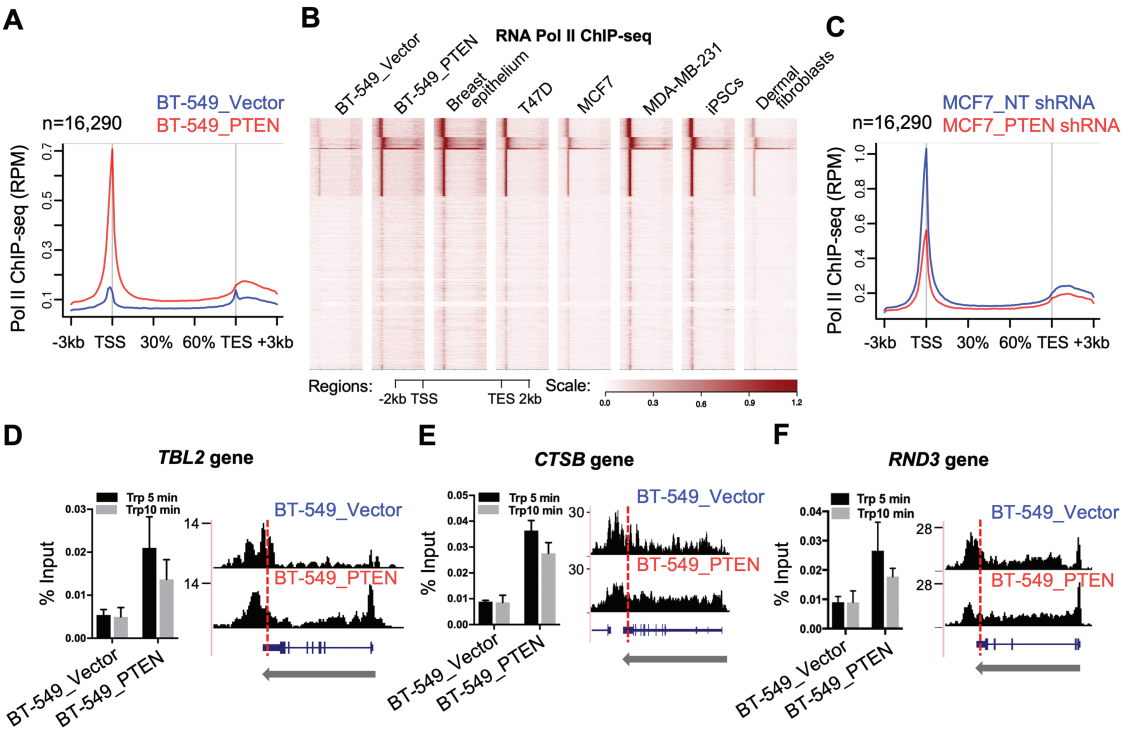

Figure 4. PTEN redistributes genome-wide Pol II occupancy. **(A)** Metagene plot showing Pol II signals on gene bodies in BT-549 cells after overexpression of PTEN followed by Pol II ChIP-seq. **(B)** Heatmap showing Pol II signals on gene bodies in various cell types (Breast epithelium, SRR2301044; T47D, SRR7965856; MCF7, SRR1290687; MDA-MB-231, SRR5919406; iPSCs, SRR5935361; and Dermal fibroblasts, SRR4340777). **(C)** Metagene plot showing Pol II signals on gene bodies in MCF7 cells after knock-down of PTEN followed by Pol II ChIP-seq. **(D)** Pol II elongation was compared after 5 and 10 min of Trp treatment (0.5 μM) followed by ChIP-qPCR for Pol II signal at the terminal exon of TBL2 gene. **(E)** Pol II elongation was compared after 5 and 10 min of Trp treatment followed by ChIP-qPCR for Pol II signal at the terminal exon of CTSB gene. **(F)** Pol II elongation was compared after 5 and 10 min of Trp treatment followed by ChIP-qPCR for Pol II signal at the terminal exon of RND3 gene. Dotted vertical red line indicates the primer position used in ChIP-qPCR.

role in transcriptional regulation, specifically in the context of cancer development and neurological disorders. For instance, understanding the roles of different *PTEN* mutations, as well as its proteoforms, in transcriptional regulation, may provide a road map for improved diagnostic and therapeutic management of PTEN-related disorders, both germline and somatic.

Materials and Methods

Cell culture and reagents

BT-549 (HTB-122™) and MCF7 (HTB-22™) cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). Both were molecularly authenticated by STRS analyses and utilized at passage range 3–6 for experiments. Cells were cultured in DMEM/F-12 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Thermo Scientific Gibco, Waltham, MA) at 37°C with 5% CO2. To prepare PTEN expressing stable lines, BT-549 (PTEN null) cells were transfected with pTet-Off (Clontech Takara, Mountain View, CA) using Fugene® 6 (Promega, Madison, WI) according to manufacturer's protocols. Multiple stable clones were isolated using neomycin selection and tested with pTRE2Hyg-Luc according to manufacturer's protocols for optimal expression and tetracycline regulation. Clone B2 was chosen and transfected using Fugene® 6 with a pTRE2Hyg (Clontech Takara) backbone containing 3X N-terminal FLAG-tagged PTEN or empty vector control. Both stable PTEN expressing and stable control clones were selected by hygromycin treatment. PTEN clones were grown in 1 ug/ml tetracycline and screened for Tet-regulated expression of PTEN according to manufacturer's protocols. Optimal clones were expanded, and earliest passages were frozen for future use. MCF7 cells were transfected using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA) with PTEN-targeting MISSION™ TRC shRNA TRCN0000002746 shRNA or the MISSION Non-targeting control SHC002 (both from Sigma-Aldrich, St. Louis, MO). Stable clones were selected by puromycin treatment and PTEN knock-down of each targeting clone determined by western blot. The clone with the best knock-down was expanded and frozen for future use. All the experiments were performed when cells were at 70–80% confluency. Triptolide (Trp) (Sigma-Aldrich, St. Louis, MO) treatment was given at the concentration of 500 nM for the given time course.

Immunoblotting

Protein lysates were prepared using the M-PER (Thermo Scientific) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich) and quantified by using BCA protein assay (Thermo Scientific). Lysates were separated by SDS-PAGE and transferred onto nitrocellulose membranes followed by probing with appropriate antibodies (see Supplementary Material for the list of antibodies). Blots were scanned digitally using the GE Amersham Imager 600 (GE Healthcare Life Science, Chicago, Illinois).

RNA isolation and RT-qPCR

Total RNA was extracted from cells using the RNeasy kit (Qiagen, Germantown, MD). On-column DNase treatment was given

following the procedure provided by the manufacturer. One microgram of RNA was used for cDNA preparation using random hexamer priming and SSRT III reverse transcriptase (Life Technologies) followed by qPCR using specific primer sets. Results were analyzed using the standard ΔΔCT method. Data were derived from at least three independent biological replicates and are shown as mean ± SEM values.

RNA-seq

RNA-seq was performed using Qiagen kit-purified RNA at the Genomics Core, Lerner Research Institute, Cleveland Clinic. RNA quality was assessed via the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). All samples have RIN numbers ≥9.5. Two independent biological replicates (each containing two technical replicates) were used for RNA-seq. The library was prepared using Illumina TruSeq stranded total RNA kit (Illumina, San Diego, CA) according to manufacturer's protocols. Sequencing was performed using Illumina HiSeq 2500 using 100 bp paired-end rapid run format.

ChIP, library preparation and sequencing

Nuclei were isolated from formaldehyde (1% w/v, methanol-free) crosslinked cells by using NEXSON method (46) followed by chromatin preparation using a Covaris sonicator (Covaris, Woburn, MA). Chromatin was immunoprecipitated using an appropriate antibody, washed, reverse-crosslinked and DNA isolated using the phenol–chloroform method. Precipitated DNA was quantified by quantitative PCR and normalized against 1% input. Each experiment was performed using a minimum of three independent biological replicates for quantitative PCR analysis and two independent biological replicates (each containing two technical replicates) for ChIP-sequencing. For ChIP-sequencing, 10 ng precipitated DNA was used to prepare library using Illumina TruSeq ChIP library preparation kit. Libraries were quantified for cluster generation using KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA). Sequencing was performed using Illumina HiSeq2500 using 50 bp paired-end rapid run format.

Data analysis

RNA-seq and ChIP-seq sequencing quality was initially checked by running FastQC. RNA-seq reads were mapped against hg19 using STAR aligner. Differential gene expression analysis was performed using DESeq2 with adjusted *P*-value <0.05 considered statistically significant. In the MCF7 RNA-seq analysis, the batch effect was modeled by using a design of '∼condition + date' in the DESeq2 program. Pathway analysis was performed using IPA (Qiagen). For ChIP-seq analysis, reads were aligned against hg19 by using Bowtie2. BAM files were normalized by depth after removal of PCR duplicates and blacklisted regions. For heatmap and metagene plots, ngs.plot program was used. GRCh37/hg19 gene list was curated after removing non-canonical, mitochondrial, genes shorter than 2 kb and overlapping genes on the same strand, which resulted in 21 396 genes. UCSC genome browser was used to visualize individual gene tracks. To calculate the Pol II PRR, first, two bed files were generated using 21 396 genes list by modifying the coordinates for promoter and gene body. Pol II ChIP-seq signals were counted by using bedcov function of samtools. PRR was calculated by taking the ratio of gene body (+300 bp to +2 kb from TSS) counts to promoter (−50 bp to +300 bp form TSS) counts. Cumulative frequency plots and box plots were plotted by using PRR.

Supplementary Material

Supplementary Material is available at *HMG* online.

Acknowledgements

We thank Eng laboratory members for assistance, helpful discussions and critical reading of the manuscript draft. C.E. is the Sondra J. and Stephen R. Hardis Endowed Chair in Cancer Genomic Medicine.

Conflict of Interest statement. None declared.

Accession number

ChIP-seq and RNA-seq data have been deposited at Gene Expression Omnibus (GEO) and are accessible through GEO accession number GSE124659.

Funding

American Cancer Society Clinical Research Professorship (to C.E.).

Author contributions

A.A. and C.E. conceptualized the study; A.A. designed experiments; A.A. and T.R. performed the experiments; A.A. and R.P. performed data analysis; and A.A. and C.E. interpreted the data analysis and drafted the manuscript. All authors critically reviewed the manuscript and gave final approval.

References

1. Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., Peacocke, M. *et al.* (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat. Genet.*, **16**, 64–67.
2. Marsh, D.J., Dahia, P.L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R.J. and Eng, C. (1997) Germline mutations in PTEN are present in Bannayan–Zonana syndrome. *Nat. Genet.*, **16**, 333–334.
3. Nieuwenhuis, M.H., Kets, C.M., Murphy-Ryan, M., Yntema, H.G., Evans, D.G., Colas, C., Moller, P., Hes, F.J., Hodgson, S.V., Olderode-Berends, M.J. *et al.* (2014) Cancer risk and genotype–phenotype correlations in PTEN hamartoma tumor syndrome. *Fam. Cancer*, **13**, 57–63.
4. Yehia, L., Ngeow, J. and Eng, C. (2019) PTEN-opathies: from biological insights to evidence-based precision medicine. *J. Clin. Invest.*, **129**, 452–464.
5. Tan, M.H., Mester, J.L., Ngeow, J., Rybicki, L.A., Orloff, M.S. and Eng, C. (2012) Lifetime cancer risks in individuals with germline PTEN mutations. *Clin. Cancer Res.*, **18**, 400–407.
6. Nelen, M.R., Padberg, G.W., Peeters, E.A., Lin, A.Y., van den Helm, B., Frants, R.R., Coulon, V., Goldstein, A.M., van Reen, M.M., Easton, D.F. *et al.* (1996) Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat. Genet.*, **13**, 114–116.
7. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R. *et al.* (1997) PTEN, a putative protein tyrosine phosphatase gene mutated

in human brain, breast, and prostate cancer. *Science*, **275**, 1943–1947.
8. Myers, M.P., Stolarov, J.P., Eng, C., Li, J., Wang, S.I., Wigler, M.H., Parsons, R. and Tonks, N.K. (1997) P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc. Natl. Acad. Sci. U. S. A.*, **94**, 9052–9057.
9. Lee, Y.R., Chen, M. and Pandolfi, P.P. (2018) The functions and regulation of the PTEN tumour suppressor: new modes and prospects. *Nat. Rev. Mol. Cell Biol.*, **19**, 547–562.
10. Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Siderovski, D.P. and Mak, T.W. (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, **95**, 29–39.
11. Correia, N.C., Girio, A., Antunes, I., Martins, L.R. and Barata, J.T. (2014) The multiple layers of non-genetic regulation of PTEN tumour suppressor activity. *Eur. J. Cancer*, **50**, 216–225.
12. Bermudez Brito, M., Goulielmaki, E. and Papakonstanti, E.A. (2015) Focus on PTEN regulation. *Front. Oncol.*, **5**, 166.
13. He, X., Saji, M., Radhakrishnan, D., Romigh, T., Ngeow, J., Yu, Q., Wang, Y., Ringel, M.D. and Eng, C. (2012) PTEN lipid phosphatase activity and proper subcellular localization are necessary and sufficient for down-regulating AKT phosphorylation in the nucleus in Cowden syndrome. *J. Clin. Endocrinol. Metab.*, **97**, E2179–E2187.
14. Chung, J.H. and Eng, C. (2005) Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. *Cancer Res.*, **65**, 8096–8100.
15. Hou, S.Q., Ouyang, M., Brandmaier, A., Hao, H. and Shen, W.H. (2017) PTEN in the maintenance of genome integrity: from DNA replication to chromosome segregation. *Bioessays*, **39**, 1700082.
16. Mansour, W.Y., Tennstedt, P., Volquardsen, J., Oing, C., Kluth, M., Hube-Magg, C., Borgmann, K., Simon, R., Petersen, C., Dikomey, E. *et al.* (2018) Loss of PTEN-assisted G2/M checkpoint impedes homologous recombination repair and enhances radio-curability and PARP inhibitor treatment response in prostate cancer. *Sci. Rep.*, **8**, 3947.
17. Shen, W.H., Balajee, A.S., Wang, J., Wu, H., Eng, C., Pandolfi, P.P. and Yin, Y. (2007) Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell*, **128**, 157–170.
18. Brandmaier, A., Hou, S.Q. and Shen, W.H. (2017) Cell cycle control by PTEN. *J. Mol. Biol.*, **429**, 2265–2277.
19. Chen, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A., Niki, M., Koutcher, J.A., Scher, H.I., Ludwig, T., Gerald, W. *et al.* (2005) Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*, **436**, 725–730.
20. Chung, J.H., Ostrowski, M.C., Romigh, T., Minaguchi, T., Waite, K.A. and Eng, C. (2006) The ERK1/2 pathway modulates nuclear PTEN-mediated cell cycle arrest by cyclin D1 transcriptional regulation. *Hum. Mol. Genet.*, **15**, 2553–2559.
21. Hopkins, B.D., Hodakoski, C., Barrows, D., Mense, S.M. and Parsons, R.E. (2014) PTEN function: the long and the short of it. *Trends Biochem. Sci.*, **39**, 183–190.
22. Planchon, S.M., Waite, K.A. and Eng, C. (2008) The nuclear affairs of PTEN. *J. Cell Sci.*, **121**, 249–253.
23. Milella, M., Falcone, I., Conciatori, F., Cesta Incani, U., Del Curatolo, A., Inzerilli, N., Nuzzo, C.M., Vaccaro, V., Vari, S., Cognetti, F. *et al.* (2015) PTEN: multiple functions in human malignant tumors. *Front. Oncol.*, **5**, 24.
24. Chen, Z.H., Zhu, M., Yang, J., Liang, H., He, J., He, S., Wang, P., Kang, X., McNutt, M.A., Yin, Y. *et al.* (2014) PTEN interacts with histone H1 and controls chromatin condensation. *Cell Rep.*, **8**, 2003–2014.
25. Benitez, J.A., Ma, J., D'Antonio, M., Boyer, A., Camargo, M.F., Zanca, C., Kelly, S., Khodadadi-Jamayran, A., Jameson, N.M., Andersen, M. *et al.* (2017) PTEN regulates glioblastoma oncogenesis through chromatin-associated complexes of DAXX and histone H3.3. *Nat. Commun.*, **8**, 15223.
26. Horita, H., Wysoczynski, C.L., Walker, L.A., Moulton, K.S., Li, M., Ostriker, A., Tucker, R., McKinsey, T.A., Churchill, M.E., Nemenoff, R.A. *et al.* (2016) Nuclear PTEN functions as an essential regulator of SRF-dependent transcription to control smooth muscle differentiation. *Nat. Commun.*, **7**, 10830.
27. Liang, H., Chen, X., Yin, Q., Ruan, D., Zhao, X., Zhang, C., McNutt, M.A. and Yin, Y. (2017) PTENbeta is an alternatively translated isoform of PTEN that regulates rDNA transcription. *Nat. Commun.*, **8**, 14771.
28. Tilot, A.K., Bebek, G., Niazi, F., Altemus, J.B., Romigh, T., Frazier, T.W. and Eng, C. (2016) Neural transcriptome of constitutional Pten dysfunction in mice and its relevance to human idiopathic autism spectrum disorder. *Mol. Psychiatry*, **21**, 118–125.
29. Duan, S., Yuan, G., Liu, X., Ren, R., Li, J., Zhang, W., Wu, J., Xu, X., Fu, L., Li, Y. *et al.* (2015) PTEN deficiency reprogrammes human neural stem cells towards a glioblastoma stem cell-like phenotype. *Nat. Commun.*, **6**, 10068.
30. Hsin, J.P. and Manley, J.L. (2012) The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes Dev.*, **26**, 2119–2137.
31. Adelman, K. and Lis, J.T. (2012) Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat. Rev. Genet.*, **13**, 720–731.
32. Samarakkody, A., Abbas, A., Scheidegger, A., Warns, J., Nnoli, O., Jokinen, B., Zarns, K., Kubat, B., Dhasarathy, A. and Nechaev, S. (2015) RNA polymerase II pausing can be retained or acquired during activation of genes involved in the epithelial to mesenchymal transition. *Nucleic Acids Res.*, **43**, 3938–3949.
33. Shen, S.M., Ji, Y., Zhang, C., Dong, S.S., Yang, S., Xiong, Z., Ge, M.K., Yu, Y., Xia, L., Guo, M. *et al.* (2018) Nuclear PTEN safeguards pre-mRNA splicing to link Golgi apparatus for its tumor suppressive role. *Nat. Commun.*, **9**, 2392.
34. Gonda, T.J. and Ramsay, R.G. (2015) Directly targeting transcriptional dysregulation in cancer. *Nat. Rev. Cancer*, **15**, 686–694.
35. Bradner, J.E., Hnisz, D. and Young, R.A. (2017) Transcriptional addiction in cancer. *Cell*, **168**, 629–643.
36. Zeitlinger, J., Stark, A., Kellis, M., Hong, J.W., Nechaev, S., Adelman, K., Levine, M. and Young, R.A. (2007) RNA polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. *Nat. Genet.*, **39**, 1512–1516.
37. Gilchrist, D.A., Fromm, G., dos Santos, G., Pham, L.N., McDaniel, I.E., Burkholder, A., Fargo, D.C. and Adelman, K. (2012) Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. *Genes Dev.*, **26**, 933–944.
38. Gilchrist, D.A., Dos Santos, G., Fargo, D.C., Xie, B., Gao, Y., Li, L. and Adelman, K. (2010) Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell*, **143**, 540–551.
39. Henriques, T., Gilchrist, D.A., Nechaev, S., Bern, M., Muse, G.W., Burkholder, A., Fargo, D.C. and Adelman, K. (2013) Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. *Mol. Cell*, **52**, 517–528.

40. Shivji, M.K.K., Renaudin, X., Williams, C.H. and Venkitaraman, A.R. (2018) BRCA2 regulates transcription elongation by RNA polymerase II to prevent R-loop accumulation. *Cell Rep.*, **22**, 1031–1039.
41. Van Oss, S.B., Cucinotta, C.E. and Arndt, K.M. (2017) Emerging insights into the roles of the Paf1 complex in gene regulation. *Trends Biochem. Sci.*, **42**, 788–798.
42. Steurer, B., Janssens, R.C., Geverts, B., Geijer, M.E., Wienholz, F., Theil, A.F., Chang, J., Dealy, S., Pothof, J., van Cappellen, W.A. *et al.* (2018) Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA polymerase II. *Proc. Natl. Acad. Sci. U. S. A.*, **115**, E4368–E4376.
43. Erickson, B., Sheridan, R.M., Cortazar, M. and Bentley, D.L. (2018) Dynamic turnover of paused Pol II complexes at human promoters. *Genes Dev.*, **32**, 1215–1225.
44. Dujardin, G., Lafaille, C., de la Mata, M., Marasco, L.E., Munoz, M.J., Le Jossic-Corcos, C., Corcos, L. and Kornblihtt, A.R. (2014) How slow RNA polymerase II elongation favors alternative exon skipping. *Mol. Cell*, **54**, 683–690.
45. Climente-Gonzalez, H., Porta-Pardo, E., Godzik, A. and Eyras, E. (2017) The functional impact of alternative splicing in cancer. *Cell Rep.*, **20**, 2215–2226.
46. Arrigoni, L., Richter, A.S., Betancourt, E., Bruder, K., Diehl, S., Manke, T. and Bonisch, U. (2016) Standardizing chromatin research: a simple and universal method for ChIP-seq. *Nucleic Acids Res.*, **44**, e67.