

Original Research

Polyploid cisplatin-resistant cancer cells have altered nuclear organization and epigenomic status

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ABSTRACT

Chemotherapy resistance remains a critical barrier in cancer treatment, partly driven by polyploid cells that survive therapy and contribute to tumor recurrence. Here, we investigated epigenomic and transcriptional changes associated with cisplatin-surviving polyploid cells compared to parental cancer cells in prostate cancer (PC3) and triple-negative breast cancer (MDA-MB-231) cell lines. We observed persistent dysregulation of chromatin compaction and altered nuclear structure in polyploid cells following cisplatin treatment. Genome-wide chromatin accessibility profiling via ATAC-seq revealed significant remodeling, notably decreased promoter accessibility at proliferation-associated loci and increased accessibility of distal regulatory elements linked to inflammation and stress response. RNA-seq analyses demonstrated a coordinated transcriptional shift away from proliferative signatures toward inflammatory and survival pathways, including activation of NFκB, interferon response, and integrated stress response pathways. Importantly, we identified subsets of genes showing concordant changes in promoter accessibility and transcriptional activity, directly linking chromatin remodeling to transcriptional reprogramming. These integrated findings highlight the role of chromatin dynamics and epigenetic plasticity in chemotherapy resistance, demonstrating that widespread chromatin accessibility changes facilitate the transition to a stress-adapted, polyploid cell state. This study provides new insights into the molecular mechanisms supporting cancer cell persistence after chemotherapy.

Introduction

Chemotherapy resistance remains a major challenge in the treatment of advanced cancers. While many tumor cells undergo cell death in response to chemotherapeutic agents such as cisplatin, a fraction of cells survive and adapt, giving rise to cell populations that drive recurrence and treatment failure [1–4]. Increasing evidence indicates that these outcomes are not solely governed by fixed genetic mutations but by non-genetic mechanisms, including transcriptional plasticity, metabolic rewiring, and chromatin remodeling [4–8].

Among these adaptive responses, progressive whole genome doubling (WGD) has been observed across a range of cancers following chemotherapy or radiation exposure. Previously, it has been shown that

cancer cells surviving genotoxic therapy undergo increases in nuclear and cellular size and enter a modified cell cycle called endocycling, characterized by repeated genome duplication without division [9]. This process plays a physiological role in normal tissues such as the liver and placenta [10–12], and its activation in cancer has been linked to stress tolerance, lineage plasticity, and tumor progression [10–13]. Additional studies have shown that such cells adapt metabolically and phenotypically to support survival and malignancy, including dysregulated iron homeostasis that sensitizes cells to ferroptosis, accumulation of lipid droplets to buffer oxidative stress, and altered cellular morphology that enhance metastatic potential [14–18]. However, despite these observations, it remains poorly understood how chromatin accessibility and transcriptional regulation are coordinated during this highly polyploid,

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therapy-adapted state, and how these regulatory changes related to the observed metabolic and phenotypic features of survival. We lack a framework for the extent successive rounds of genome duplication causally drives stress tolerance versus merely correlating with these traits.

To address these gaps, we interrogate epigenomic and transcriptomic changes in endocycling cancer cells that survive chemotherapy to identify widespread changes in accessibility that may underlie transcriptional reprogramming. Using PC3 (prostate) and MDA-MB-231 (breast) cancer cell lines, we performed ATAC-seq and RNA-seq across multiple post-treatment time points to assess how the chromatin accessibility landscape is remodeled in cisplatin-surviving cancer cells. We integrated these separate datasets to identify transcriptional programs and regulatory networks that distinguish endocycling survivors from their parental counterparts.

We demonstrate that cisplatin-surviving cells exhibit sustained chromatin decompaction, altered histone modification profiles, and widespread remodeling of promoter accessibility, particularly at loci involved in proliferation, genome maintenance, and stress signaling. Integration of chromatin accessibility and transcriptomic datasets reveals a coordinated shift in chromatin accessibility and gene expression, marked by the loss of proliferative programs and the emergence of inflammatory and stress-response signatures. These findings begin to define how chromatin accessibility and gene expression are co-regulated in non-proliferative, highly polyploid, cisplatin-resistant cancer cells and provide a framework for dissecting the mechanisms that sustain this therapy-adapted state.

Methods and materials

Cell culture

PC3 and MDA-MB-231 cell lines were maintained in RPMI-1640 with L-glutamine (PC3) or DMEM with D-glucose, L-glutamine, and sodium pyruvate (MDA-MB-231; Gibco). All media was supplemented with 10 % FBS, 1 % penicillin and streptomycin.

Cisplatin dose response curves were obtained via alamarBlue assay (VWR, #76285-554), and median lethal doses [LD50] for PC3 (6 μ M) and MDA-MB-231 (12 μ M) cell lines were calculated. Sub-confluent cultures of PC3 or MDA-MB-231 cells were treated with [LD50] cisplatin and cultured for 72 hours. Following treatment, cells were rinsed with PBS and cultured with standard media until timepoint indicated.

ATAC-seq data processing and peak calling

ATAC-seq sample and library prep are described in Supplemental Methods. Raw sequencing reads were demultiplexed and trimmed for adapter sequences using Cutadapt (v1.18; with parameter *-minimum-length 40*). Quality control of raw reads was performed using FastQC (v0.12.1). Reads were aligned to the GRCh38 human reference (patch release 14, NCBI accession GCA_000001405.29) using Bowtie2 (v2.3.5.1) with parameters: *-no-unal -no-discordant -threads 14 -X 2000*. Duplicate reads were flagged with Picard. Technical replicates were merged, and duplicated reads, mitochondrial reads, and low-quality alignments were removed using Samtools (v1.6). Library complexity for each merged sample was determined via ATACseqQC (v1.29.0) in R (v4.4.1) and libraries were normalized based on the least-complex biological replicate through subsampling.

Accessible chromatin peaks were called using Genrich (v0.6.2) in ATAC-seq mode (*-j* flag) with standard parameters; blacklisted GRCh38 genomic regions were excluded from analysis. To assess reproducibility, peak calling was performed on each biological replicate. A consensus peak set across all samples was created from peaks present in 2 or more samples.

Peak annotation

Peaks were annotated to genomic features using ChIPseeker (v1.40.0) and the GRCh38 gene annotation from TxDb.Hsapiens.UCSC.hg38.knownGene (v3.2.2). Peaks located within promoter regions (± 1 kb from the transcription start site) were categorized as promoter-proximal, while others were classified as 5' UTR, 3' UTR, 1st exon, other exon, 1st intron, other intron, downstream, and distal intergenic. Promoter-proximal peaks were annotated to the nearest gene for downstream functional analyses.

Differential accessibility analysis

Read counts were quantified across the consensus peak set in R using featureCounts from the Rsubread package (v3.19), followed by normalization; differential accessibility analysis was performed with DESeq2 (v1.44.0). Peaks with an adjusted p-value (*padj*) < 0.05 and an $|\log_2\text{FoldChange}| > 0.5$ were considered differentially accessible. To generate a set of differentially accessible genes (rather than peaks/genomic regions) per comparison, annotated peaks were first subsetted to those in promoter-proximal regions only.

Enrichment analyses

Gene ontology enrichment analysis was performed on significant (adjusted p-value < 0.05) promoter-proximal subset of differentially accessible peaks for each group of each comparison with enrichGO() from clusterProfiler (v4.14.6). For genes for which there were multiple annotated peaks, statistics from the peak with the most significant adjusted p-value were taken forward as the statistics for that gene. Gene set enrichment analysis was performed on this subset of significantly differentially accessible promoter genes using fgsea (v1.30.0) to identify enriched biological pathways in each sample compared to each other sample. Genes associated with differentially accessible peaks were ranked by their $\log_2$ fold changes, and enrichment was tested against hallmark gene sets from the MSigDB database. Pathways with an FDR-adjusted p-value < 0.05 were considered significantly enriched.

HOMER transcription factor motif enrichment analysis

Differential accessibility results for each comparison were subsetted to those peaks for which *padj* < 0.01 and $|\log_2\text{fold change}| > 0.5$ and converted to BED file format. HOMER ("Hypergeometric Optimization of Motif Enrichment"; v4.11.1) analysis was performed with the following parameters: *findMotifsGenome.pl -size 200 -mask*. Enrichment of known transcription factor motifs was determined as compared to a set of randomly selected background sequence regions.

RNA-seq analysis

ATAC-seq sample, library prep, and quality control are described in Supplemental Methods. Differential expression analysis for single-cell data was performed using the Mann-Whitney U test. UCell scoring (v2.12) was performed on the single-cell RNA-seq data set to determine gene signatures in single-cell datasets. Differential expression analysis was performed on the pseudobulked samples with DESeq2 (v1.44.0). Genes with an adjusted p-value (*padj*) < 0.05 and an $|\log_2\text{FoldChange}| > 0.5$ were considered differentially expressed, and PCA/sample clustering was performed to assess the overall variance between conditions.

Western blotting

Cells were lysed in ice-cold lysis buffer [8 M urea, 1 % SDS, 50 mM Tris-HCl pH 7.5, 150 mM NaCl, protease inhibitor cocktail (added fresh)] on ice for 1 h, passed through a 27½ gauge needle 4x, and

centrifuged at 4°C for 15 min at 12,000 rpm to remove cell debris. Protein was resolved via SDS-PAGE and transferred onto nitrocellulose membranes. After blocking in casein buffer, membranes were incubated with primary antibodies followed by secondary antibodies for detection (see Table S1 for antibodies). Blots imaged on a LICOR Odyssey and quantified by Image Studio Lite software.

Immunofluorescence imaging

24 hours prior to each timepoint, cells were seeded onto 12 mm round glass coverslips (Fisherbrand, #12541001). Cells were fixed in 10 % formalin for 15 min, permeabilized for 20 min in 0.3 % Triton-X-100, and blocked with 10 % goat serum for 1 h. Samples were incubated with DAPI (Invitrogen, #D3571) for 1 h at room temperature and mounted with ProLong™ Diamond Antifade Mountant (Invitrogen, # P36970). Images were acquired using a Zeiss Observer Z1 microscope/ZEN pro

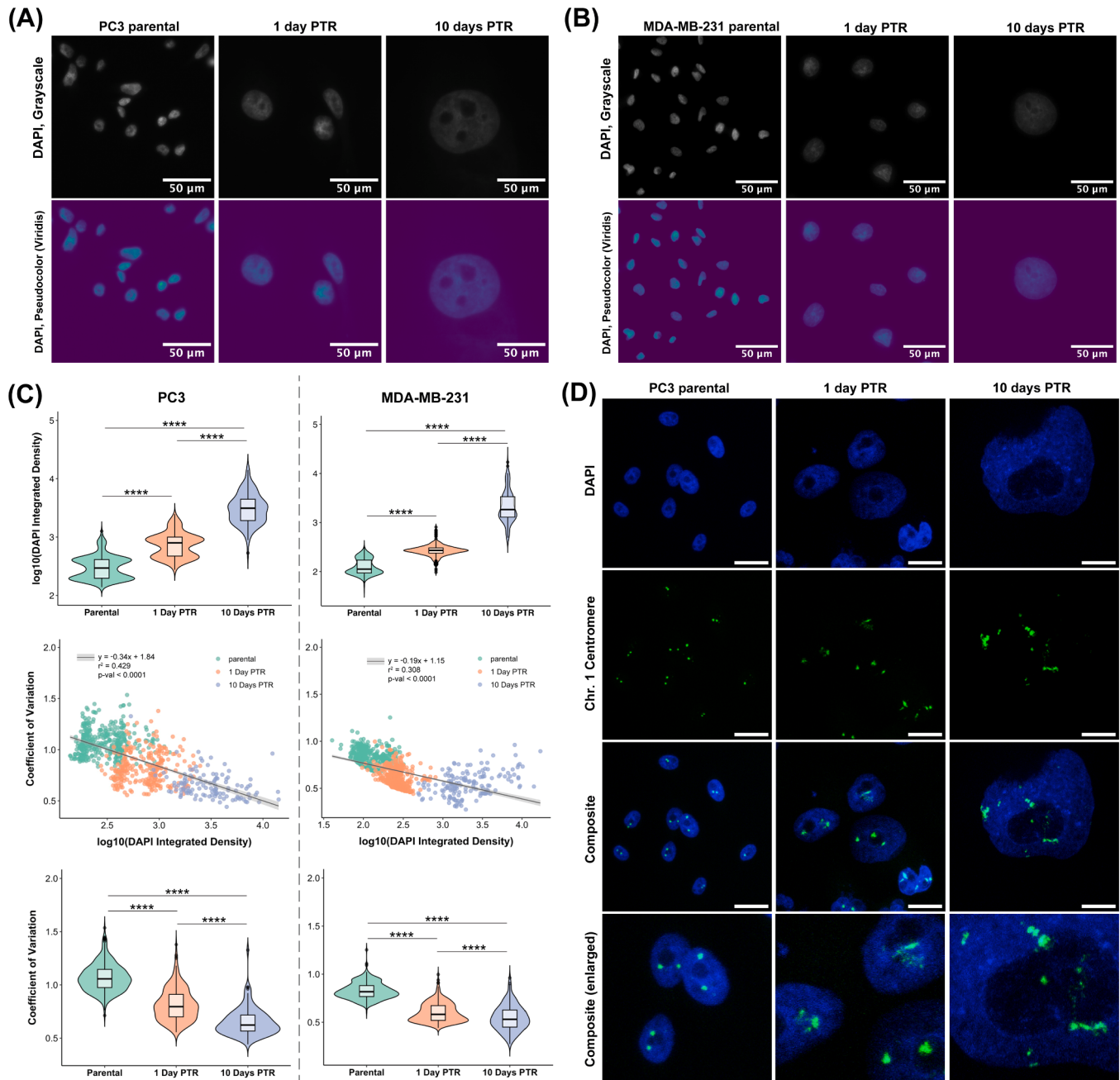


Fig. 1. Altered nuclear structure and reduced chromatin compaction in cisplatin-surviving cancer cells. Control and cisplatin-surviving were fixed and stained with DAPI to visualize chromatin. (A) Grayscale and pseudocolored images of the nuclei of parental PC3 cells without cisplatin treatment and of cells that survive 72 hours cisplatin treatment, 1 and 10 days PTR. (B) Grayscale and pseudocolored images of the nuclei of parental MDA-MB-231 cells without cisplatin treatment and of cells that survive 72 hours cisplatin treatment, 1 and 10 days PTR. (C) DAPI integrated density of each treatment group (top), coefficient of variation (CV) of DAPI signal plotted against the DAPI integrated density (middle), and comparison of DAPI CV between treatment groups (bottom) in PC3 and MDA-MB-231 parental cells and cells 1 and 10 days PTR. For (C), a difference between groups was determined using a one-way ANOVA, with post-hoc Tukey's multiple comparisons test to detect between-group differences. P-values reported as <0.0001 (****). (D) DNA FISH for the centromeric region of chromosome 1 in parental PC3 cells and cisplatin-surviving cells 1 and 10 days PTR.

2.0 software (Carl Zeiss Microscopy).

Chromatin compaction analysis

Chromatin compaction was quantified using a custom-built Python pipeline. Briefly, a Gaussian adaptive threshold was used to segment individual nuclei. Nuclei that overlapped image edges were removed. Average and normalized intensity of each nucleus in each image were calculated. Normalized intensity was scaled between 0 and 255. Coefficient of variation for each nucleus was calculated by dividing the normalized mean intensity by the normalized standard deviation.

DNA FISH

DNA FISH of chromosome 1 was performed using Vysis FISH Pre-treatment Reagent Kit (Abbott, #02J03-032) and Empire Genomics Con 1 Probe Set (Empire Genomics, #CHR01-10-GR) with a modified manufacturer's protocol. Cells were cultured on 12 mm glass coverslips before fixation in ice-cold Carnoy's fixative (3:1 Methanol:Glacial acetic acid) for 30 min. Coverslips were washed in 1X PBS, incubated in 2X SSC buffer for 2 min at 73 °C, placed onto glass microscope slide with 8 µl of FISH probe mixture (1.6 µL Chr1 probe: 6.4 µL hybridization buffer), sealed with rubber cement, denatured in an oven at 72 °C for 2 min, and hybridized at 37 °C overnight. Following hybridization, coverslips were removed from the slides, placed in 24-well plate, washed with 0.3 % NP-40 in 0.4x SSC for 2 min at 73 °C, then washed at room temperature with 0.1 % NP40 in 2x SSC for 1 min. Samples were counterstained with DAPI for 10 min, mounted on glass slides with ProLong™ Diamond Antifade Mountant. Images were acquired with a laser scanning confocal microscope (AX R, Nikon Instruments) equipped with a tunable GaAsp detector, 2 K resonant scanner, and LUA-S6 laser unit, and Apochromat Lambda S LWD 40XC water immersion N.A. 1.15 objective (MRD77410, Nikon Instruments).

Results

Cisplatin-surviving cancer cells exhibit altered nuclear structure and reduced chromatin compaction

Prostate cancer cell line PC3 and breast cancer cell line MDA-MB-231 were treated with LD50 doses of cisplatin, 6 µM and 12 µM respectively, for 72 hours. Cultures were washed and the surviving cells were allowed to recover in fresh, complete media up to 10 days post treatment release (PTR).

We observed an increase in nuclear size in cells PTR in both cell lines (Fig. 1A, B, C), consistent with previously reported data [9]. When we quantified the DAPI signal, we saw the expected relative 2N/4 N population in the mitotic control cells and a progressive increase in DNA content in days PTR, indicating that cells 10 days PTR are highly polyploid (well beyond tetraploid) compared to their parental counterparts, and have undergone multiple endocycles/WGDs (Fig. 1C). The nuclear DAPI coefficient of variation (CV; a ratio of the standard deviation to the mean of the intensity values) can be utilized as a proxy for the relative degree of chromatin compaction [19–21]. A higher CV represents a higher degree of chromatin compaction while a lower CV represents the inverse. We observed that the CV decreases as total DNA content increases in all groups (Fig. 1C). DAPI CV was significantly lower in surviving cells 1 d and 10 days PTR in both cell lines, suggesting that the degree of chromatin compaction is lower in these treatment groups compared to treatment-naïve cells (Fig. 1C).

We also examined chromatin decompaction via DNA FISH for the centromeric region of chromosome 1. PC3 parental cells had compact focal centromere staining, with approximately 2 foci/nucleus (Fig. 1E). Cisplatin-treated cells showed less focal staining, an effect most pronounced in cells 10 days PTR, which had diffuse, smear-like staining (Fig. 1E). Taken together, the DAPI CV and DNA FISH analyses indicate a

progressive chromatin decompaction indicative of long-term changes in nuclear organization post-chemotherapy.

Histone modifications and chromatin-associated proteins are dysregulated after cisplatin treatment

To investigate the molecular underpinnings of the observed chromatin decompaction, we examined changes in levels of histone modifications and key chromatin-associated proteins. We observed decreased total H3 levels at 1 d and 10 days PTR in both PC3 and MDA-MB-231 (Fig. 2A–D). H3K9me3 is a repressive histone modification [22–25] that is deposited by histone methyltransferases including EHMT2 (G9a) and SUV39H1. Relative H3K9me3/total H3 is slightly increased 1 d PTR but not at 10 days PTR compared to controls (Fig. 2A). EHMT2 and SUV39H1 were reduced at 1 and 10 days PTR in both cell lines (Fig. 2A). H3K27me3 is a repressive histone modification catalyzed by the methyltransferase EZH2 [26–28]. BMI1 is recruited to H3K27me3-marked chromatin and promotes chromatin compaction [29–32]. We observed no change in relative H3K27me3/total H3 levels but decreases in levels of both EZH2 and BMI1 (Fig. 2B).

Histone acetylation is generally associated with transcriptional activation and chromatin decompaction [33–35]. HDAC1 is a class I histone deacetylase that removes acetyl groups from lysine residues on histones, thereby promoting chromatin compaction [36–38]. Relative levels of H3K9ac and H3K14ac to total H3 were unchanged in both cell lines (Fig. 2C, D). While HDAC1 levels were significantly decreased 1 and 10 days PTR in PC3s, this decrease was not observed in MDA-MB-231 cells (Fig. 2C).

HP1α is a central heterochromatin-associated protein that binds to methylated H3K9 and facilitates heterochromatin compaction through chromatin looping and recruitment of silencing machinery [39–41]. In PC3 cells, there was a slight decrease in HP1α levels between day 1 and days 10 PTR. In MDA-MB-231 cells, there was an increase in HP1α expression between parental cells and day 1 PTR, which returned to control levels at 10 days PTR (Fig. 2E).

Widespread chromatin accessibility remodeling occurs in post-cisplatin cells, reflecting a shift to increased accessibility of distal regulatory elements

We performed ATAC-seq to investigate how these changes in nuclear architecture and chromatin compaction impact the global chromatin accessibility landscape in cisplatin-surviving cells. Due to limitations of existing droplet-based single cell ATAC-seq technologies and the large size of the cells, this was performed on a bulk level. We first examined the genomic distribution of ATAC-seq peaks across different functional elements. In all groups, peaks were most frequently located within distal intergenic and intronic regions, with relatively fewer peaks in promoter regions (± 1 kb from the TSS), untranslated regions, or exonic sequences (Fig. 3A, B). Across gene bodies, we observed a sharp enrichment of ATAC-seq peaks immediately at the TSS, consistent with accessible promoter regions, and a broader, lower signal downstream of gene bodies (Fig. 3A, B). The overall pattern of chromatin accessibility at both genic and intergenic features remained qualitatively similar between parental and cells that survive cisplatin treatment.

We next identified regions of differential chromatin accessibility between untreated control cells and cells that survived cisplatin treatment (Table S2). Peaks with adjusted p-value < 0.05 and $|\log_2\text{Fold-Change}| > 0.5$ were considered differentially accessible (DA). 88,102 peaks in PC3 and 69,933 peaks in MDA-MB-231 were DA between control and cells 10 days PTR.

The untreated groups had more peaks with increased accessibility in promoter-proximal regions (22.66 % in PC3 and 29.44 % in MDA-MB-231) compared to cells 10 days PTR (5.06 % and 6.2 %), while more of the peaks with increased accessibility in cells 10 days PTR fell in distal intergenic regions (Fig. 3C, D). In both cell lines, the same trend was

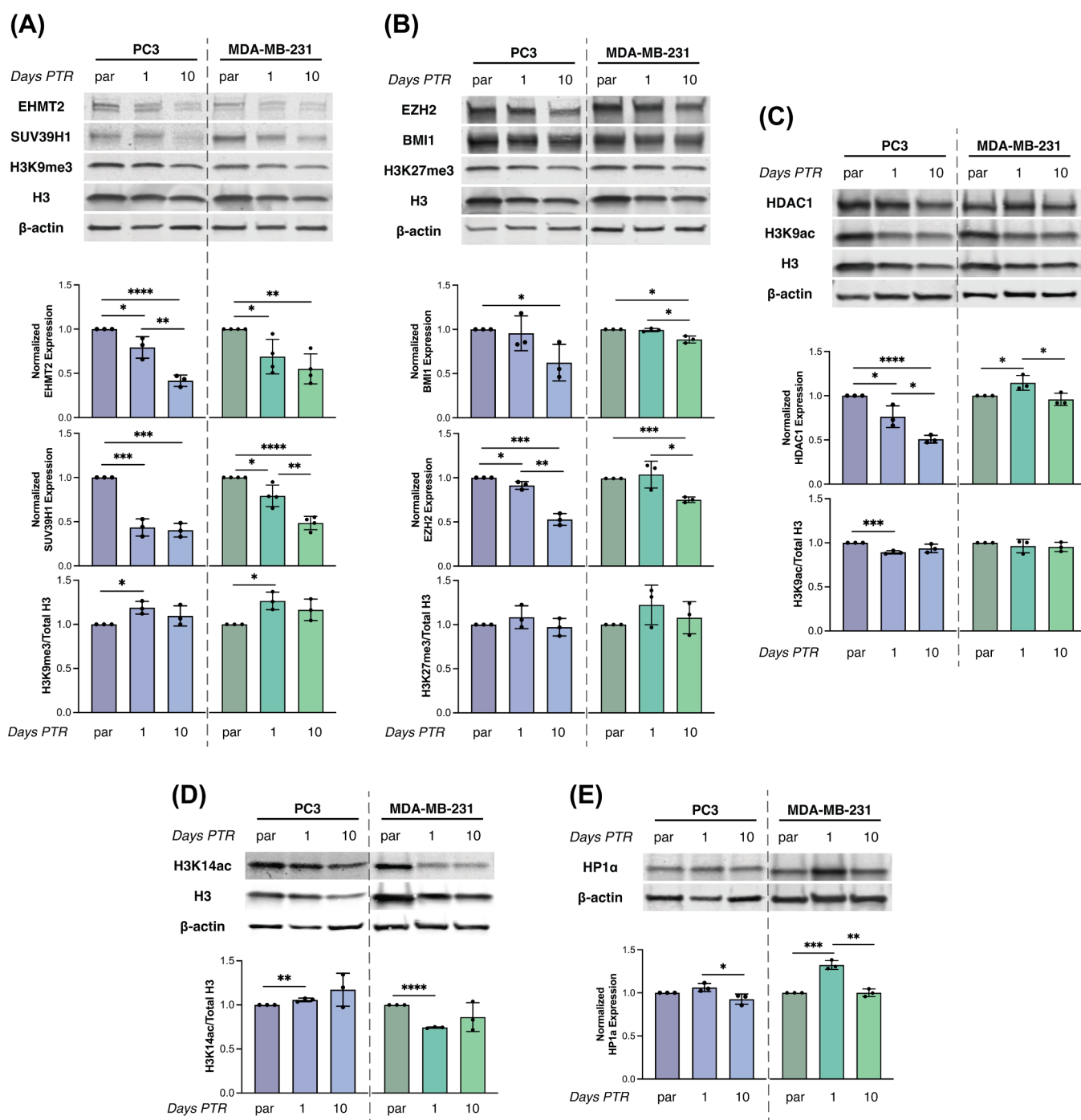


Fig. 2. Levels of histone modifications and chromatin-associated proteins are dysregulated in cisplatin-surviving cancer cells. (A) Western blots for EHMT2, SUV39H1, and H3K9me3 in PC3 and MDA-MB-231 cells without cisplatin treatment and in cells that survive 72 hours cisplatin treatment, 1 and 10 days PTR. Quantification of relative expression levels (via densitometry) in biological replicates is shown below the blots. (B) Western blots and quantification for EZH2, SUV39H1, and H3K27me3 in PC3 and MDA-MB-231 cells without cisplatin treatment and in surviving cisplatin-treated cells 1 and 10 days PTR. (C) Western blots and quantification for HDAC1 and H3K9ac in PC3 and MDA-MB-231 cells without cisplatin treatment and in surviving cisplatin-treated cells 1 and 10 days PTR. (D) Western blots and quantification for H3K14ac in PC3 and MDA-MB-231 cells without cisplatin treatment and in surviving cisplatin-treated cells 1 and 10 days PTR. (E) Western blots and quantification for HP1 α in PC3 and MDA-MB-231 cells without cisplatin treatment and in surviving cisplatin-treated cells 1 and 10 days PTR. P-values are reported as <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****).

observed in the comparisons between parental and 1 d PTR samples (Fig. S1A, B). These results suggest a shift toward increased accessibility of distal regulatory elements in cisplatin-surviving cells, potentially reflecting a reorganization of transcriptional control mechanisms in the polyploid, treatment-adapted state.

Promoter accessibility shifts away from proliferative gene programs in treatment-surviving cells

To investigate how altered promoter accessibility might influence overall transcriptional programming, we analyzed the subset of DA peaks annotated to promoter regions. Promoter-proximal peaks were annotated to the nearest gene for downstream functional analyses. The

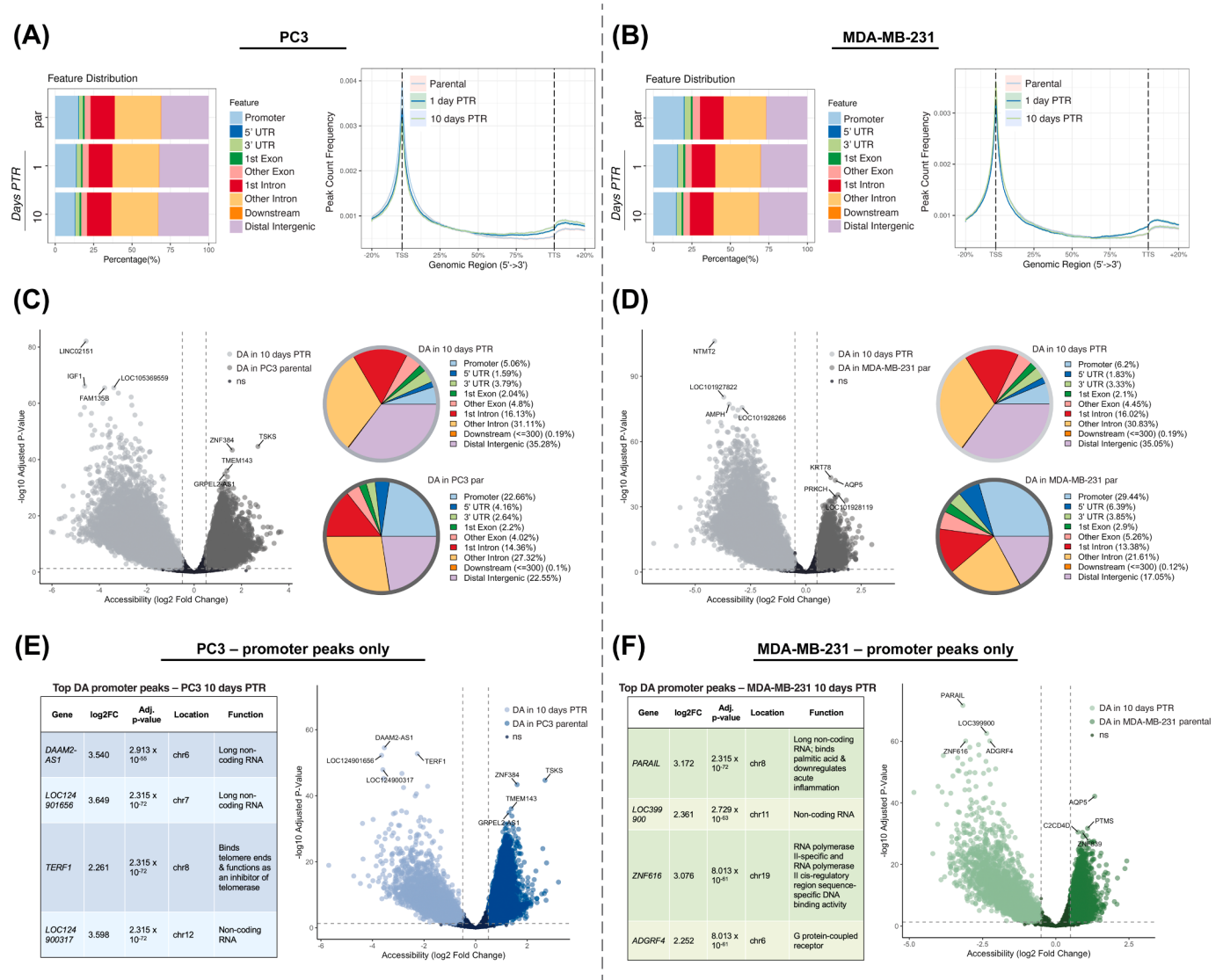


Fig. 3. Shifts in chromatin accessibility of distal intergenic regions in cisplatin-surviving cancer cells. ATAC-sequencing was performed on PC3 and MDA-MB-231 cells without cisplatin treatment and in cells that survive 72 hours cisplatin treatment, 1 and 10 days PTR (A, B) Distribution of ATAC-seq peaks across functional genomic elements and gene bodies in cells without cisplatin treatment and in cells that survive 72 hours cisplatin treatment, 1 and 10 days PTR, in PC3 and MDA-MB-231 cells, respectively. (C, D) Differential accessibility analysis was performed using DESeq2 to compare ATAC-seq peaks in untreated cells and surviving cisplatin-treated cells 10 days PTR in PC3 and MDA-MB-231 cells, respectively. Top differentially accessible (DA) peaks for each group as indicated on the volcano plots were annotated to the closest downstream gene. Horizontal dashed line represents significance cutoff of $p_{adj} < 0.05$. Vertical dashed lines represent $|\log_2 \text{fold change}| > 0.5$. Annotations shown to the right of the volcano plots only include differentially accessible (DA) peaks that meet the cutoff thresholds. (E, F) DA peaks subsetted only to those that fall within promoter regions, for PC3 and MDA-MB-231 cells, respectively. Functional role of top DA promoter peaks in cells 10 days PTR is indicated.

top DA peaks in PC3 parental cells compared to cells 10 days PTR mapped to *TSKS*, *ZNF384*, *TMEM143*, and *GRPEL2-AS1*, while top peaks in cells day 10 PTR mapped to *DAAM2-AS1*, *LOC124901656*, *TERF1*, and *LOC124900317*, implicating cytoskeletal remodeling, telomere maintenance, and potential regulatory lncRNAs (Fig. 3E). In MDA-MB-231 s, *AQP5*, *PTMS*, *ZNF839*, and *C2CD4D* were DA in parental cells and *PARIL*, *LOC399900*, *ZNF616*, and *ADGRF4* were DA in cells 10 days PTR, pointing to transcription factors, lncRNAs, and G-protein signaling (Fig. 3F). Similar trends were observed in comparisons between parental and 1 d PTR in both cell lines (Fig. S2A, B). These results suggest that cisplatin-surviving cells favor promoter accessibility of genes linked to adaptive stress responses, genome stability, and non-canonical regulatory networks.

Functional enrichment and motif analysis reveal activation of stress- and inflammation-associated regulators

To probe the functional impact of changes in promoter-proximal accessibility, we performed gene ontology enrichment for DA promoter-linked genes. In parental cells, DA promoter peaks were enriched for essential biosynthetic and genome maintenance processes, including ribonucleoprotein complex biogenesis, DNA replication, and double-strand break repair, while cells 10 days PTR DA promoter peaks were enriched for genes relating to altered cell morphology and non-canonical lipid and ion signaling (Fig. 4A, B). The same trend was observed in between parental and 1 d PTR samples (Fig. S3A, B). Although specific enriched ontologies differed between cell lines, both indicate a shift from canonical proliferative and repair programs toward alternative signaling, homeostatic regulation, and stress response. These findings suggest that promoter remodeling following cisplatin treatment

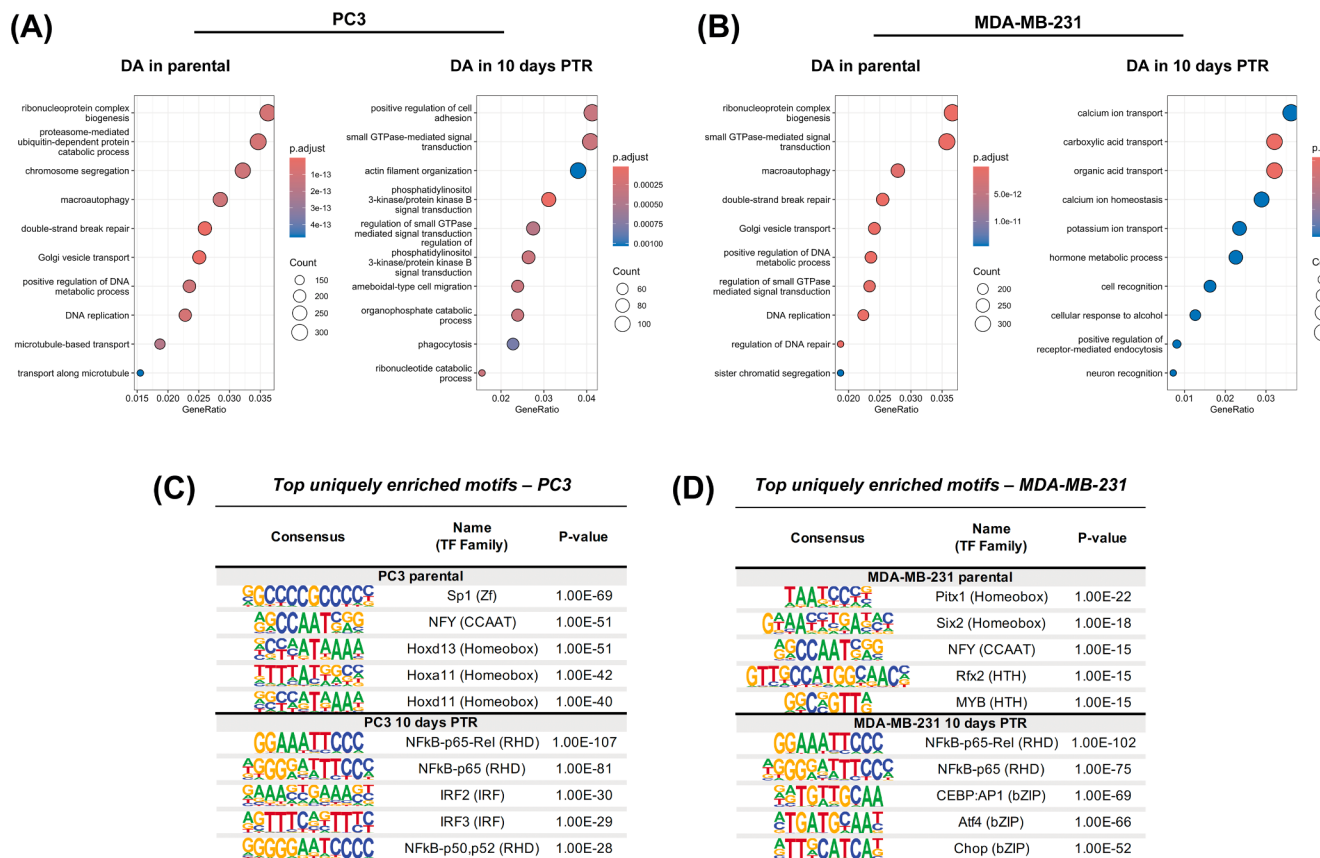


Fig. 4. Gene ontology and transcription factor motif enrichment in cisplatin-surviving cancer cells. (A) Gene ontology enrichment analysis for the significant ($\text{padj} < 0.05$) subset of DA promoter peaks annotated to the nearest downstream gene for each group in the comparison between untreated PC3 parental cells and cisplatin-surviving PC3 cells 10 days PTR. (B) Gene ontology enrichment analysis for the significant ($\text{padj} < 0.05$) subset of DA promoter peaks annotated to the nearest downstream gene for each group in the comparison between untreated MDA-MB-231 parental cells and MDA-MB-231 cells 10 days PTR. (C, D) Motif enrichment analysis was performed on the set of significant DA peaks ($\text{padj} < 0.01$ and $|\log_2 \text{fold change}| > 0.5$) for each group in the comparison between untreated parental cancer cells and cisplatin-surviving cells 10 days PTR. The top uniquely enriched motifs for each group in (C) PC3 and (D) MDA-MB-231 are reported.

may reflect a shared epigenetic adaptation that enables long-term survival in cisplatin-surviving cells.

We performed a transcription factor motif enrichment analysis on the significant DA peaks. The most enriched motifs for both parental cells and cells 10 days PTR in both cell lines were AP-1 family transcription factors (Fig. S4). When examining the top enriched motifs *unique to each group* (parental vs. 10 days PTR), we observed consistent activation of transcription factors involved in inflammation and cellular stress responses in PC3 and MDA-MB-231 cells 10 days PTR (Fig. 4C, D). Both cell lines showed strong enrichment in motifs for NFκB-p65, NFκB-p65-Rel, and NFκB-p50,p52, key mediators of pro-inflammatory and survival signaling. In PC3 cells, additional enrichment for IRF2 and IRF3 suggests activation of interferon-related transcriptional programs (Fig. 4C). In MDA-MB-231 cells, motifs for CEBP:AP1, ATF4, and CHOP were enriched, indicating engagement of integrated stress response and ER stress pathways (Fig. 4D). Similar motif enrichment results were found in cells 1 d PTR compared to parental in both cell lines (Fig. S5A, B). These findings suggest that transcription factor motif accessibility in cisplatin-surviving cells is shaped by inflammatory and stress-related transcriptional regulators commonly associated with damage response.

RNA-seq reveals transcriptional reprogramming promoting survival in post-cisplatin cells

To determine whether changes in chromatin accessibility were reflected at the transcriptional level, we performed RNA sequencing. Single-cell RNA sequencing was performed via combinatorial barcoding to accommodate cell size. We used unsupervised dimensionality

reduction to identify transcriptomic signatures (Fig. S6), plotted clusters using Uniform Manifold Approximation and Projection (UMAP), and performed UCell enrichment scoring.

Untreated PC3 cells clustered independently from cisplatin-surviving cells (Cluster 5), while treated cells occupied several clusters, indicating a heterogeneous, dynamic response to chemotherapy (Fig. 5A, B). Cells 1 d PTR predominated Clusters 0 and 2, while cells 10 days PTR made up the majority of Cluster 4; cells 5 days PTR were the most represented in Cluster 1, but were also spread throughout Clusters 0, 2, 3, and 4 (Fig. 5A, B). All clusters containing cells post-cisplatin treatment were enriched for gene sets related to EMT, RIG-I-like signaling, and TNFA signaling (Fig. 5E). Compared to Cluster 5, de-enrichment of genes associated with E2F targets, DNA replication, and ribosome biogenesis also defined the clusters predominated by cisplatin-surviving cells (Fig. 5E).

Untreated parental MDA-MB-231 cells did not cluster completely independently, but rather the cluster also contained day 10 PTR cells (Cluster 0, Fig. 5C, D). Cells 1 d PTR clustered primarily in Clusters 2 and 5, and all post-cisplatin cells occupied overlapping clusters (Clusters 1, 3, 4, 6). Similar to the PC3 cell line, clusters predominated by post-cisplatin MDA-MB-231 cells were defined by gene signatures related to EMT and TNFA signaling and were de-enriched for gene sets relating to ribosome production, DNA replication, and the G2M checkpoint (Fig. 5F). For both cell lines, similar trends in UCell scoring were observed when examining the scRNA-seq data by treatment group rather than cluster identity, revealing distinct signatures in cells that survive cisplatin treatment (Fig. S7; Fig. S8).

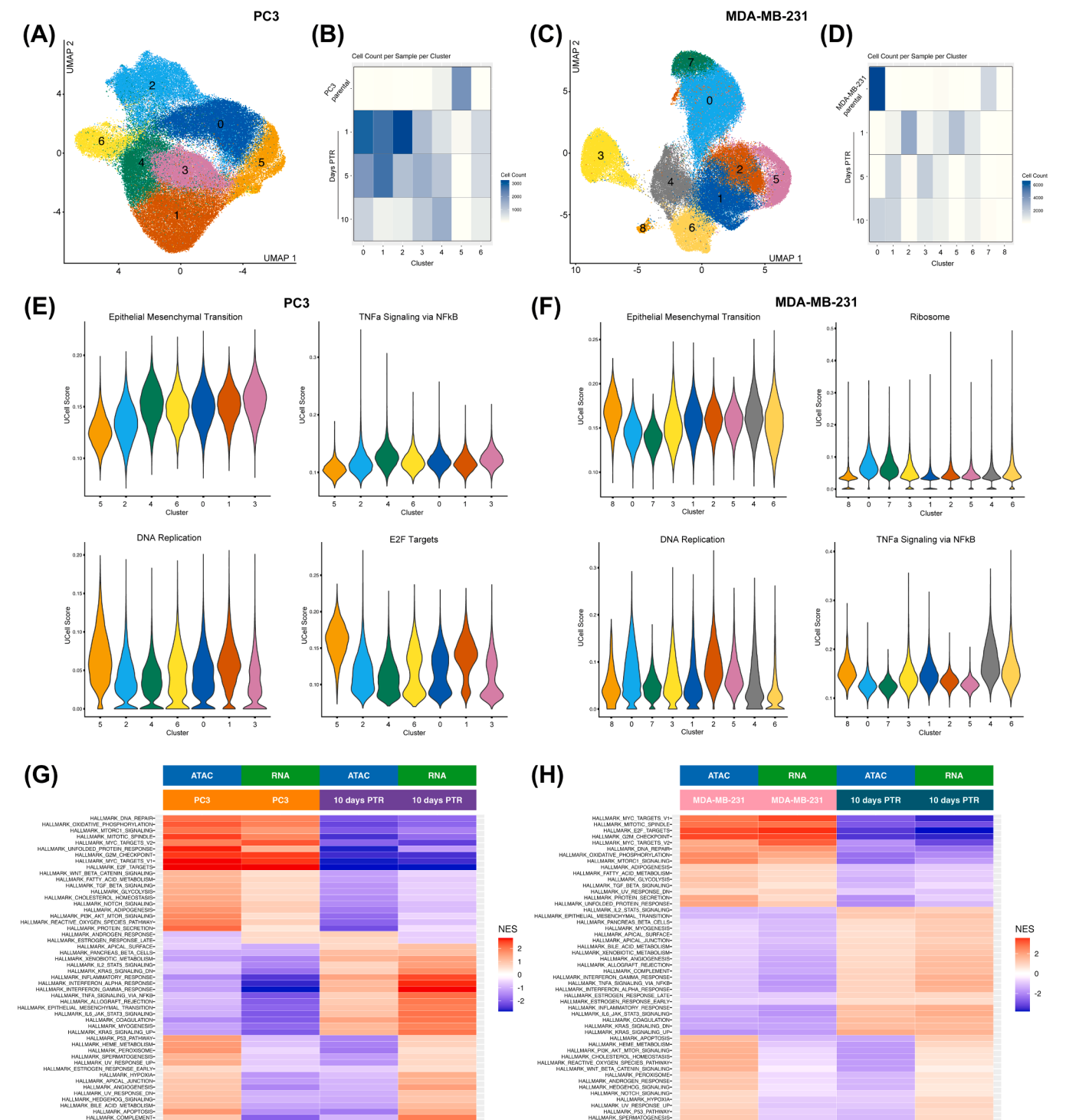


Fig. 5. Transcriptional reprogramming promotes survival in cisplatin-surviving cancer cells. Single-cell RNA sequencing was performed using a combinatorial barcoding method to accommodate the large size of cells 10 days PTR. (A) Uniform Manifold Approximation and Projection (UMAP) for scRNA-seq dataset containing untreated PC3 cells and cisplatin-surviving PC3 cells 1, 5, and 10 days PTR. (B) Distribution of samples across clusters in (A). (C) UMAP for scRNA-seq dataset containing untreated MDA-MB-231 cells and cisplatin-surviving MDA-MB-231 cells 1, 5, and 10 days PTR. (D) Distribution of samples across clusters in (C). (E) UCell enrichment scoring of selected gene sets across each cluster in (A). (F) UCell enrichment scoring of selected gene sets across each cluster in (C). (G, H) Heatmaps comparing hallmark gene set enrichment (via GSEA) for the DA promoter-proximal genes from the ATAC-seq data and differential gene expression (DGE) results from the RNA-seq data, for the parental and 10 days PTR samples in the PC3 and MDA-MB-231 cell lines, respectively. For genes in the ATAC-seq data for which there were multiple annotated peaks, statistics from the peak with the most significant adjusted p-value were taken forward as the statistics for that gene. To better align the transcriptomic data with the bulk chromatin accessibility profiles obtained from ATAC-seq with our transcriptional data, the RNA-seq data was pseudo-bulked across cells within each group prior to the DGE analysis.

ATAC-seq and RNA-seq data independently indicate enrichment of inflammatory and stress response-related genes

We next investigated the relationship between chromatin accessibility and gene expression. To align the transcriptomic data with the bulk chromatin accessibility profiles from ATAC-seq, we pseudo-bulked the RNA-seq data across cells within each group (parental, day 1, and 10 days PTR) and performed differential gene expression (DGE) analysis. We collapsed the promoter-mapping ATAC-seq data for genes with multiple annotated peaks to obtain a single set of DA statistics per gene;

statistics from the peak with the most significant adjusted p-value were taken forward. We performed GSEA of the Hallmark gene sets on these DA promoter-proximal genes and DGE data for each treatment group of each cell line to assess whether changes in chromatin accessibility were reflected in transcriptional programs (Fig. 5D, F). In PC3 and MDA-MB-231 d 10 PTR cells, we observed enrichment of gene sets related to inflammatory and stress signaling, and de-enrichment of those related to DNA repair, MYC target gene expression, E2F targets, and cell cycle (Fig. 5D, F). The same trends were observed in the comparisons between parental and 1 d PTR samples (Fig. S9A, B). Many of the most

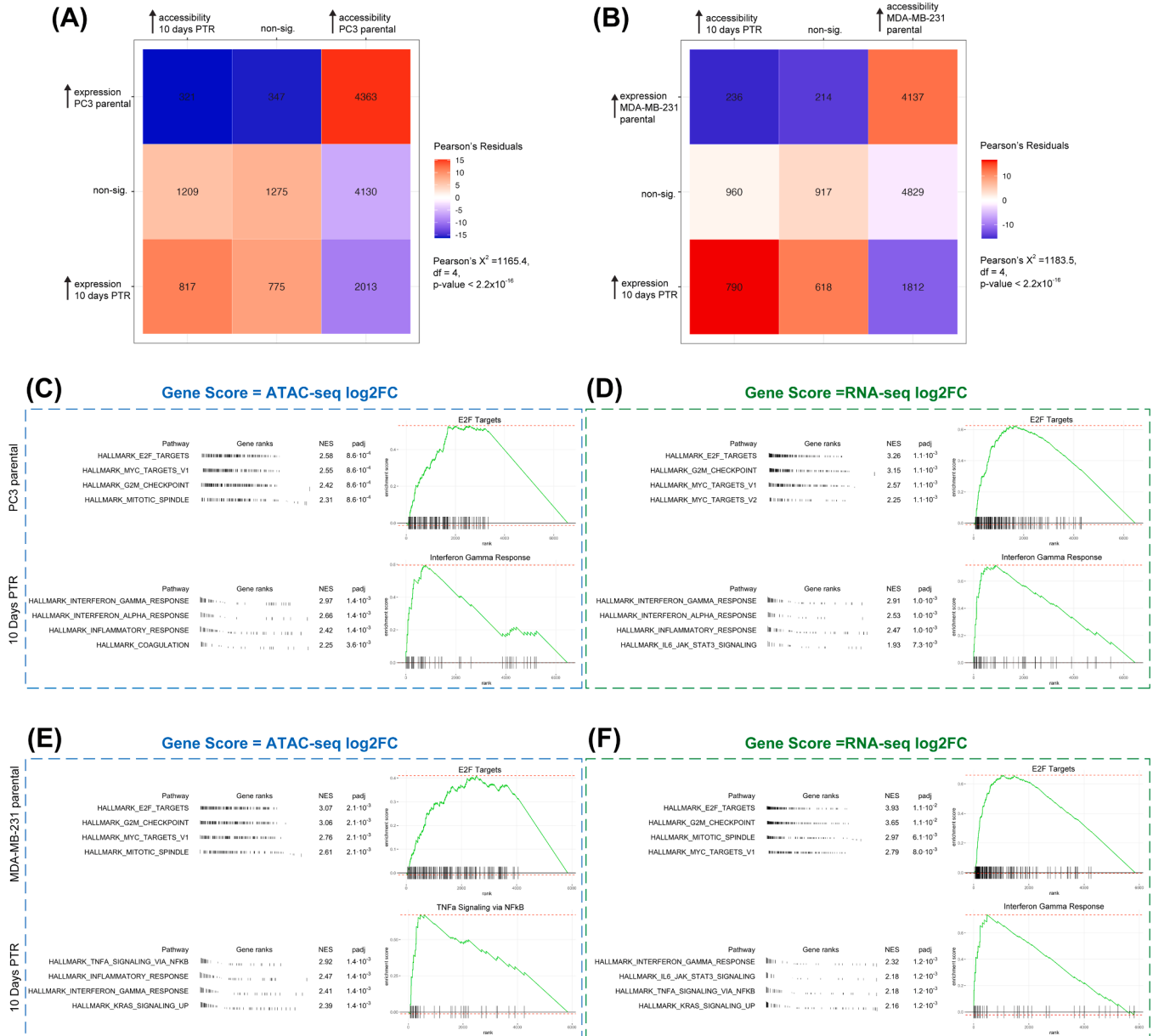


Fig. 6. Chromatin accessibility and transcriptional changes in cisplatin-surviving cancer cells converge on shared regulatory programs. (A) Contingency table to test for a significant, non-random association between promoter-proximal chromatin accessibility and differential gene expression in the comparison between untreated PC3 parental cells and cisplatin-surviving PC3 cells 10 days PTR. The set of genes represented is those present both in the results from the collapsed, promoter-annotated DA gene list referenced in Fig. 5G and in the DGE analysis results for the pseudo-bulked RNA-seq data. For both ATAC-seq and RNAseq, significance is defined as $\text{padj} < 0.05$ and $|\log_2 \text{fold change}| > 0$. Pearson's Chi-squared test was used to examine the association between datasets. (B) Contingency table to test for a significant, non-random association between promoter-proximal chromatin accessibility and differential gene expression in the comparison between untreated MDA-MB-231 parental cells and cisplatin-surviving MDA-MB-231 cells 10 days PTR. The set of genes was prepared similarly to in (A). (C, D) GSEA of hallmark gene sets performed on the gene list that represents the intersection of the ATAC-seq and RNA-seq data (genes where the significance status and direction of change were the same in both datasets) for untreated PC3 cells compared to cisplatin-surviving PC3 cells 10 days PTR. Either the ATAC-seq log2FoldChange (C) or RNAseq log2FoldChange (D) was used to rank the input genes. (E, F) GSEA of hallmark gene sets performed on the gene list that represents the intersection of the ATAC-seq and RNA-seq data for untreated MDA-MB-231 cells compared to cisplatin-surviving MDA-MB-231 cells 10 days PTR.

significantly altered gene sets showed consistent directionality across modalities, supporting the hypothesis that changes in chromatin accessibility at promoters contributes to transcriptional reprogramming in cisplatin-surviving cells.

Chromatin accessibility and gene expression changes converge on shared regulatory programs

Lastly, we sought to more precisely define gene sets whose transcriptional changes may be directly driven by altered promoter accessibility. We identified genes with concordant directionality and statistical significance in both ATAC-seq and RNA-seq datasets. Pearson's Chi-squared test confirmed a significant, non-random association between chromatin accessibility and differential gene expression ($X^2 = 1165.4$, $df = 4$, $p < 2.2 \times 10^{-16}$) in PC3 control versus day 10 PTR (Fig. 6A). A similar trend was observed for the comparison between MDA-MB-231 parental cells and cells 10 days PTR ($X^2 = 1183.5$, $df = 4$, $p < 2.2 \times 10^{-16}$) (Fig. 6B). A non-random association between chromatin accessibility and differential gene expression was also present between parental and cells 1 d PTR in both cell lines (Fig. S10A, B).

We performed GSEA on the genes where the significance status and direction of change were the same in the ATAC-seq and RNA-seq datasets. For PC3 and MDA-MB-231 cells, E2F targets was the most highly enriched gene set in parental cells versus cells 10 days PTR when using either the Log2 fold change from the ATAC-seq experiment or the RNA-seq dataset to rank the input genes (Fig. 6C-F). Other gene sets that were enriched in parental cells included G2M checkpoint, mitotic spindle, and MYC targets. The most significantly enriched gene sets in the cells 10 days PTR compared to parental cells in both cell lines were related to inflammation: interferon gamma response, inflammatory response, TNF α signaling via NF κ B, and IL6/JAK/STAT3 signaling (Fig. 6C-F). In both cell lines the GSEA results for the overlapping genes between the ATAC-seq and RNA-seq datasets for parental vs. cells 1 d PTR closely mirrored the enrichments observed in the parental vs. 10 days PTR comparison (Fig. S10C-F).

Together, these findings demonstrate a significant and coordinated relationship between promoter accessibility and gene expression, with untreated parental cells exhibiting enrichment of proliferative gene programs, and cisplatin-surviving cells showing a marked shift toward inflammatory and stress-responsive transcriptional states. These integrated chromatin and transcriptional changes form the basis for further interpretation of how nuclear remodeling may support survival and adaptation in polyploid cells following chemotherapy treatment.

Discussion

Together, these data reveal a consistent epigenomic and transcriptional trajectory across two genetically distinct cancer cell lines following survival to cisplatin treatment. Despite differences in tissue origin and baseline chromatin state, both PC3 and MDA-MB-231 cells 10 days PTR exhibited similar patterns of chromatin decompaction, promoter accessibility loss at proliferation-related genes, and activation of inflammation-associated transcription factors. These shared features suggest a convergent epigenetic program associated with survival in the post-treatment state and raise the possibility that chromatin relaxation and inflammatory reprogramming may be generalizable hallmarks of therapy-adapted cancer cells.

These findings extend prior studies linking DNA reorganization to cancer cell plasticity, survival, and drug resistance. WGD and chromatin decompaction have been observed in multiple models of therapy-induced persistence and stress adaptation, often accompanying cell cycle exit and genome instability [42–45]. Prior work has shown that chemotherapy can induce transient states of cell cycle exit and epigenetic relaxation, enabling re-entry into the cell cycle or emergence of resistant subclones [44–47]. This study builds upon this framework by showing that chromatin decompaction is functionally linked to durable

transcriptional reprogramming in highly polyploid, cisplatin-surviving cells that have undergone multiple rounds of endocycling, not just in near-tetraploid cells that have undergone only one WGD. In both the PC3 and MDA-MB-231 models, we observe widespread loss of promoter accessibility at genes involved in proliferation, DNA replication, and mitotic control, mirrored by reduced expression of these same gene sets in cisplatin-surviving cells. Concurrently, accessible promoters and elevated gene expression levels in post-treatment cells are enriched in inflammatory signaling, cytokine response, and cellular stress adaptation pathways. These observations demonstrate that chromatin remodeling contributes to transcriptional shifts in chemotherapy-surviving cells and may underlie their persistence in the face of genotoxic damage. Supporting this shift, motif enrichment analysis of differentially accessible promoters in cells 10 days PTR revealed increased accessibility for binding motifs of key stress- and inflammation-associated transcription factors, such as NF κ B-p65, IRFs, ATF4, and CHOP, reinforcing the engagement of damage response and adaptive regulatory programs in cisplatin-surviving cells.

Several limitations of this study warrant consideration. Our studies were limited to the treatment of cancer cell lines with only cisplatin and did not consider other chemotherapies. ATAC-seq could only be performed on bulk populations due to current cell-size limitations of single cell ATAC-seq technologies, which may obscure heterogeneity in chromatin accessibility landscapes among individual cells, an effect that may be especially relevant in the context of a polyploid, transcriptionally diverse population. While the integration of our chromatin accessibility data with single-cell RNA-sequencing offers some additional resolution, studies using single-cell ATAC-seq would enable the discernment of subpopulation-specific changes. Additionally, this study primarily focused on correlations between changes in chromatin accessibility and gene expression, and while the observed concordance is strong, we do not test causality or functional outcomes of specific chromatin changes. Functional validation of candidate chromatin regulators or stress-responsive genes will be critical to determine which chromatin changes are essential for survival and which are consequences of an altered cellular state. Finally, we focused the analyses within this study primarily to DA promoter regions, and while these show the strongest relationship to transcription, changes in accessibility at enhancers and distal regulatory elements also contribute to gene regulation and should be explored [48–50].

Future work should aim to define the functional roles of the transcription factors and chromatin regulators implicated in this study. Targeted manipulation or inhibition of chromatin-modifying enzymes such as EHMT2, EZH2, or HDAC1 may clarify whether epigenetic repression is actively maintained or passively lost in cisplatin-surviving cells. Modulation of inflammation-associated transcriptional programs or stress-response genes could reveal whether these shifts are required for cell persistence following treatment release, immune evasion, or escape from a mitotic cell cycle. Longitudinal studies will also be important to determine the stability and reversibility of these chromatin and transcriptional changes, and whether they represent transient adaptive states or fixed epigenetic transitions. Ultimately, these insights may inform strategies to eliminate persistent cell populations or prevent the emergence of resistant clones.

In summary, our findings demonstrate that alterations in chromatin state are a defining feature of cancer cells that survive cisplatin treatment, and these changes are linked to transcriptional reprogramming toward a stress-adapted, polyploid state. This work highlights the epigenetic plasticity of cisplatin-surviving cancer cells and reiterates the importance of nuclear architecture and chromatin dynamics in shaping functional transcriptional cellular responses to genotoxic stress.

CRediT authorship contribution statement

Anna LK Gonye: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis,

Conceptualization. **Linda Orzolek:** Methodology, Investigation. **Christopher Cherry:** Visualization, Formal analysis. **Michael Patatanian:** Visualization, Formal analysis. **Luke V Loftus:** Visualization, Investigation. **Kevin Truskowski:** Methodology, Investigation. **George Butler:** Software, Resources, Methodology. **Kenneth J Pienta:** Writing – review & editing, Funding acquisition, Conceptualization. **Sarah R Amend:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.neo.2025.101268](https://doi.org/10.1016/j.neo.2025.101268).

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