Received: 25 July 2017 | Revised: 20 September 2017 | Accepted: 20 September 2017

DOI: 10.1002/gcc.22505

RESEARCH ARTICLE

WILEY

Trichostatin A preferentially reverses the upregulation of gene-expression levels induced by gain of chromosome 7 in colorectal cancer cell lines

Floryne O. Buishand[1,2] | Eric Cardin[1] | Yue Hu[1] | Thomas Ried[1]

[1]Genetics Branch, Center for Cancer Research, National Cancer Institute, Building 50, Room 1408, Bethesda, Maryland

[2]Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, Utrecht, The Netherlands

Correspondence
Floryne O. Buishand, DVM, PhD; Center for Cancer Research, National Cancer Institute, Building 50, Room 1408, Bethesda, MD 20892-8010.
Email: floryne.buishand@nih.gov

Funding information
The Netherlands Organization for Scientific Research, Grant Number: 190.2015.2.310.005

Epithelial cancers are defined by a tumor-specific distribution of chromosomal aneuploidies that are maintained when cells metastasize and are conserved in cell lines derived from primary tumors. Correlations between genomic copy number and gene expression have been observed for different tumors including, colorectal (CRC), breast, and pancreatic cancer. These ploidy-driven transcriptional deregulations are characterized by low-level expression changes of most genes on the affected chromosomes. The emergence of these aberrations at an early stage of tumorigenesis and the strong selection for the maintenance of these aneuploidies suggest that aneuploidy-dependent transcriptional deregulations might contribute to cellular transformation and maintenance of the malignant phenotype. The histone deacetylase inhibitor (HDACi) Trichostatin A (TSA) has anticancer effects and is well known to lead to large-scale gene-expression changes. Here we assessed if TSA could disrupt the aneuploidy-driven gene expression in the aneuploid colon cancer cell line SW480 and the artificially generated aneuploid cell line DLD-1 + 7. We found that TSA increases transcriptional activity throughout the genome, yet inhibits aneuploidy-induced gene-expression changes on chromosome 7. Among the TSA affected genes on chromosome 7, we identified potential CRC oncogenes. These experiments represent the first attempt to explain how histone acetylation affects aneuploidy-driven gene-expression changes.

1 | INTRODUCTION

Epithelial cancers are defined by a specific distribution of chromosomal aneuploidies, that is, chromosome numbers that are not the multiple of the diploid complement.[1–3] The resulting genomic imbalances are tumor specific, are maintained when cells metastasize, and are conserved in cell lines derived from primary tumors.[4] For instance, trisomy of chromosome 7 in colorectal adenomas is one of the earliest chromosomal alterations observed in the development of sporadic colorectal carcinomas (CRCs).[5,6] During CRC carcinogenesis, the gain of chromosome 7 is later complemented by copy number increases of chromosomes and chromosome arms 13, 8q, and 20, and losses of 4 and 18.[7] These imbalances in CRC result in ploidy-driven transcriptional deregulation of genes residing on the aneuploid chromosomes and are also found in other tumor entities.[8–11]

The strong selection for the maintenance of chromosomal aneuploidies very convincingly suggests a functional relevance as drivers of tumorigenesis. However, it remains unknown to which extent, and how, aneuploidy-dependent transcriptional deregulation contributes to cellular transformation, in particular at early stages of tumorigenesis when these aberrations emerge, and to which extent they are required for the maintenance of the malignant phenotype.

The histone deacetylase inhibitor (HDACi) trichostatin A (TSA) is a well-known anticancer agent that leads to large-scale gene-expression changes and exerts its effect by altering the transcriptional regulation of specific cancer-related genes.[12] TSA inhibits HDACs in a noncompetitive and reversible way and is able to inhibit proliferation and induce differentiation in different types of cancer cells such as CRC, prostate, neuroblastoma, and skin cancer cells.[13–16] Although TSA's molecular mechanisms for inhibiting proliferation and inducing differentiation have been widely studied, it remains unknown whether TSA preferentially influences transcriptional activity on aneuploid chromosomes. To address this question, we conducted a systematic exploration of the consequences of histone modification by TSA on CRC cell lines, both

diploid and aneuploid. Of special interest was the question whether TSA could revert the gene-expression changes induced by the introduction of extra copies of chromosome 7 into the karyotpypically stable CRC cell line DLD-1.

2 | MATERIALS AND METHODS

2.1 | Cell lines and treatments

Human colon adenocarcinoma cell lines DLD-1 and SW480 were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in 10% fetal bovine serum (FBS) (Gibco, Thermofisher Scientific, Waltham, MA) supplemented RPMI (Gibco) or DMEM (Gibco) medium, respectively. DLD-1 cells harboring an extra copy of chromosome 7 (DLD-1 + 7) were previously described[9] and maintained in 10% FBS supplemented RPMI medium with 100 μg/ml geneticin (G418) (Thermofisher). Cells were seeded 24 hours prior to treatment in a 96-well flat clear bottom black plate (Corning, Corning, NY), after which they were treated with different concentrations of TSA (Sigma-Aldrich, St. Louis, MO) for 24 hours. DMSO (Sigma-Aldrich) was used as vehicle control.

2.2 | Viability assays

Cell viability was analyzed using CellTiter-Blue Assay (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, 20 μL of CellTiter-Blue was added to each well and incubated at 37°C in the dark for 90 min. Fluorescence generated by the conversion of the substrate by living cells was measured using a microplate reader SpectraMaxM2e (Molecular devices, Sunnyvale, CA) at excitation 560 nm and emission 590 nm. Viability was calculated as [experimental fluorescence of treated cells/fluorescence of control cells × 100].

2.3 | Western blots

Cells were lysed in RIPA buffer (Cell Signaling Technology, Danvers, MA). Equal amounts of protein were subjected to SDS-PAGE on precast polyacrylamide gels (Thermo Fisher Scientific), blotted onto PVDF membrane (EMD Millipore, Billerica, MA), and incubated with primary antibodies Anti-Ac-histone H3 (Abcam, Cambridge, MA), anti-Ac-histone H4 (Abcam), anti-cleaved-PARP #9541 (Cell Signaling Technology), and anti-GAPDH #G8795 (Sigma-Aldrich) as loading control. Respective secondary antibodies linked to HRP antirabbit #2004 and antimouse #2005 (SantaCruz Biotechnology, Santa Cruz, CA) were used and detection was performed by chemiluminescence using SuperSignal West Pico (Thermo Fisher Scientific).

2.4 | Gene-expression microarrays

Total RNA was isolated from the cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and RNA integrity (RIN) was assessed with an RNA 6000 Nano LabChip Kit using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Only samples with a RIN number >8.0 were included, and five replicates per condition were used. One microgram of RNA from each cell line was amplified and labeled using the Quick Amp Labeling Kit, one-color (Agilent) and subsequently hybridized on Human GE 4x44K v2 Microarrays (Agilent) according to the manufacturer's protocol version 6.5. Slides were scanned with a microarray scanner G2565BA (Agilent). Images were analyzed and data were quality controlled using Feature Extraction software version 10.7.1.1 (Agilent). The microarray specifications and derived data are accessible through National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) accession number GSE100705.

2.5 | Data analysis

Array data were $\log_2$-transformed, normalized, and corrected for multiple testing. Significance analysis for microarrays (SAM) software was used for the identification of differences in gene expression due to treatment with TSA at a false discovery rate (FDR) of 5%, with minimum fold changes of 3.0. For the identification of differently expressed gene due to the introduction of an additional copy of chromosome 7 in DLD-1 cells, a *t* test was used with *P* value <0.05 and a minimum fold change of 1.5.

IPA software (v01–10, Ingenuity, Mountain View, CA) was used to assess the involvement of significantly differentially expressed genes in known pathways. The IPA pathways were listed by significance rank order, that is, with the pathways having a lower likelihood that the generation of the pathways was serendipitous listed on top. Cutoff values for significant meaningful pathways was set at *P* value <0.05 and false discovery rate (FDR) < 0.05.

3 | RESULTS

3.1 | TSA treatment reduces viability of CRC cell lines

The treatment effect of different TSA concentrations on the viability of the diploid CRC cell line DLD-1, the cell line DLD-1 + 7 into which we introduced an extra copy of chromosome 7, and the aneuploid cell line SW480, which carried gains of chromosome 7, was analyzed. The viability of the cell lines was reduced between 30% and 60% for all concentrations tested (40–1,000 nM) (Fig. 1A). The effect of TSA on the action of histone deacetylases was assessed by Western blot analysis using antibodies against acetylated histones H3 and H4 (Fig. 1B). Acetylation of both histones was increased in a dose-dependent manner and can be clearly observed starting at 120 nM in all cell lines. The effect of TSA on apoptosis was also assessed by Western blot analysis using antibodies against cleaved PARP (Fig. 1C). Cleavage of PARP was increased in a dose-dependent manner and can be clearly observed starting at 320 nM in all cell lines. As 160 nM TSA represented the concentration that maximally inhibited histone deacetylases, while at the same time having a minimal effect on the induction of apoptosis, this concentration was used to treat the cells for gene-expression profiling.

3.2 | Effect of TSA on whole chromosome average gene-expression levels

The consequences of TSA treatment on gene expression were measured by global gene-expression profiling. The average gene expression

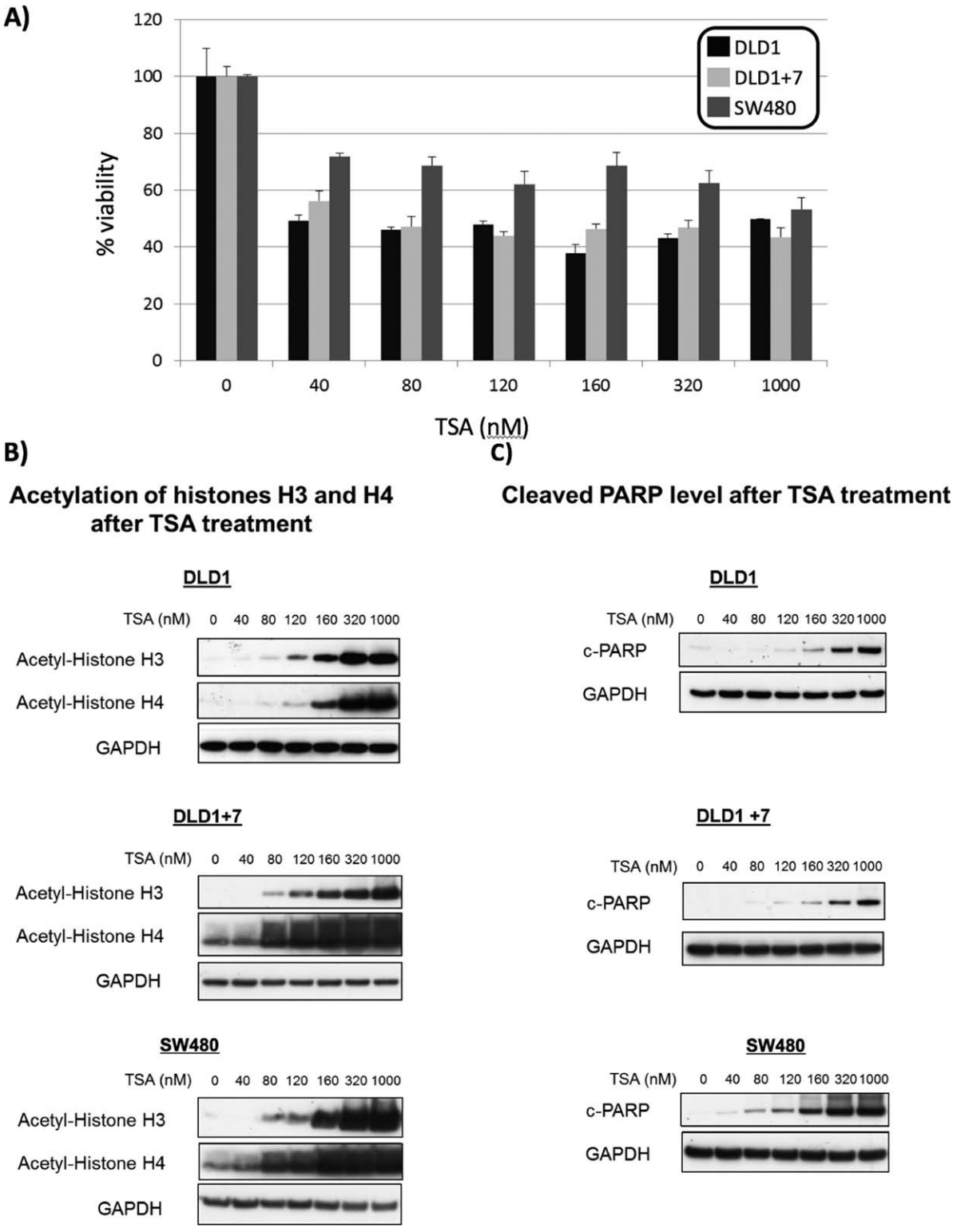

FIGURE 1 TSA treatment of colon cancer cell lines DLD-1, DLD-1 + 7, and SW480. A, Viability after treatment with different concentrations of TSA. Data represent mean % viability ± S.E.M. B, Western blot analysis with antiacetylated histone H3 and histone H4 antibodies. GAPDH antibody was used as loading control. C, Western blot analysis with cleaved-PARP (c-PARP) antibody. An antibody against GAPDH was used as loading control

of each chromosome increased in TSA treated DLD-1 and SW480 cells compared to untreated controls (Figure 2). The mere addition of an extra copy of chromosome 7 in DLD-1 cells increased overall gene expression in DLD1 + 7 on most chromosomes, except for chromosomes 11, 16, 17, 19, 20, and 22. The overall gene expression demonstrated the highest increase on chromosome 7 in DLD1 + 7 versus

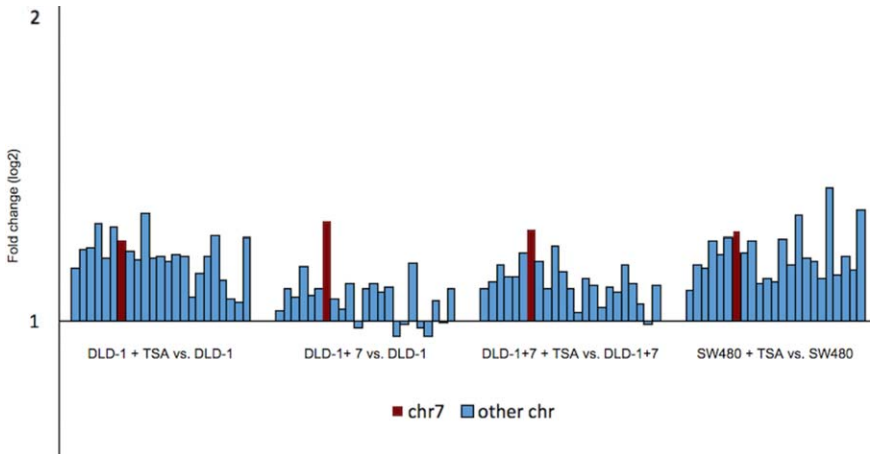

FIGURE 2 Effect of TSA and/or addition of chromosome 7 on whole-chromosome average gene expression of the DLD-1, DLD-1 + 7, and SW480 cell lines. Each bar represents the average fold change (log 2) for a chromosome as compared to its control. Chromosomes are ordered from left to right (chromosome 1–22 + chromosome X). Chromosome 7 is labeled in red [Color figure can be viewed at wileyonlinelibrary.com]

DLD1. Treatment of DLD1 + 7 cells with TSA further increased the gene-expression levels on all chromosomes, except for gene expression on chromosome 22. Overall, treatment with TSA did not preferentially affected whole-chromosome gene expression.

3.3 | Significant differential gene expression

Introduction of chromosome 7 in DLD-1 resulted in significant upregulation of 561 genes and downregulation of 258 genes throughout the genome (Supporting Information, Table 1A,B). Treatment of DLD-1 with TSA resulted in significant upregulation of 641 genes and downregulation of 171 genes (Supporting Information, Table 2A,B). The treatment effect of TSA on DLD-1 + 7 was more pronounced with 1,343 significantly upregulated and 636 downregulated genes (Supporting Information, Table 3A,B). In SW480 cells, TSA treatment resulted in significant upregulation of 1,770 genes and downregulation of 811 genes (Supporting Information, Table 4A,B).

In all three cell lines, chromosome 7 was among the top-three chromosomes with the highest percentage of significantly upregulated genes and lowest percentage of downregulated genes due to TSA treatment (Fig. 3). Interestingly, when the percentages of downregulated genes per chromosome in DLD-1 + 7 + TSA versus DLD-1 + 7 were compared to DLD-1 + TSA versus DLD-1, there was a 12-fold higher downregulation of the genes on chromosome 7 in DLD-

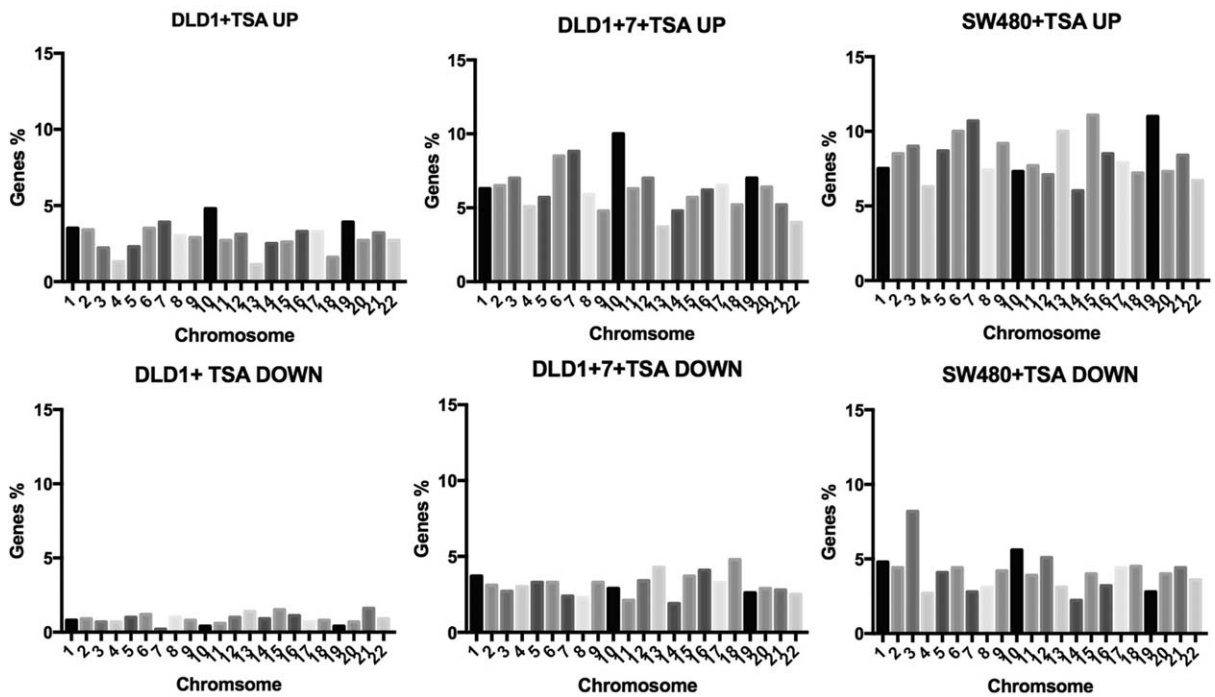

FIGURE 3 Percentages of upregulated and downregulated genes on individual chromosomes due to TSA treatment

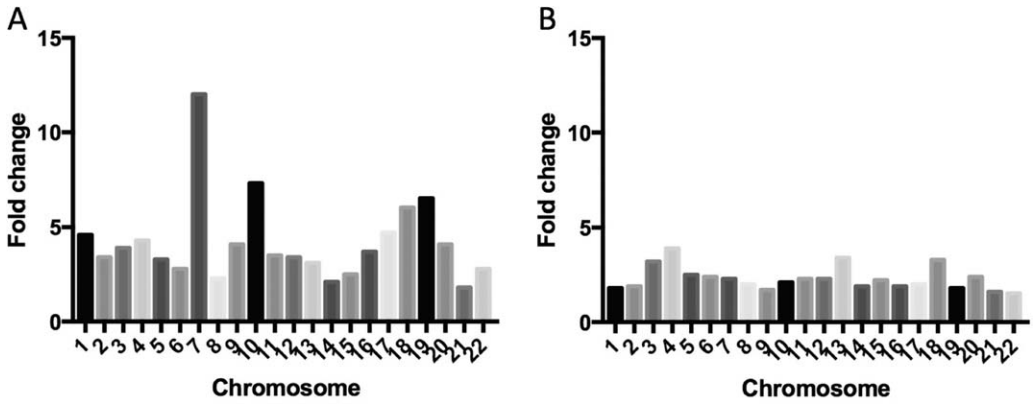

FIGURE 4 Comparison of the gene expression levels significantly changed by TSA per chromosome in DLD-1 + 7 versus DLD-1. A, Fold changes of the significant downregulated genes per chromosome in DLD-1 + 7 versus DLD-1. B, Fold changes of the significant upregulated genes per chromosome in DLD-1 + 7 versus DLD-1

1 + 7 + TSA (Fig. 4A). This highlights that TSA asserts a preferential downregulating effect on the transcriptome of chromosome 7 when an additional copy of chromosome 7 is present, compared to its downregulating effect on the other chromosomes.

3.4 | Potential therapeutic targets

To identify genes located on chromosome 7 potentially having a beneficial effect on CRC tumor cell viability, the gene list of upregulated genes in DLD-1 + 7 versus DLD-1 was compared to the gene list of downregulated genes in DLD-1 + 7 + TSA versus DLD-1 + 7. These gene lists only had three genes in common: *PON2*, *ASB4*, and *ZNF273*. Out of these genes, *ASB4* was also among the genes that were downregulated in SW480 by TSA treatment.

To identify whether the genes downregulated by TSA treatment in cells with an additional copy of chromosome 7 clustered together in gene signaling pathways, we used ingenuity pathway analysis (IPA). The following pathways were significantly downregulated in aneuploid DLD-1 + 7 and SW480 cells, but not in diploid DLD-1 cells: "Role of CHK proteins in cell cycle checkpoint control," "Estrogen-mediated S-phase entry," "Cyclins and cell cycle regulation," "Cell cycle: G1/S checkpoint regulation," and "Molecular mechanisms of cancer." The "Role of CHK proteins in cell cycle checkpoint control" and "Molecular mechanisms of cancer" pathways contained genes located on chromosome 7: *RFC2*, *PRKAR1B*, *SMO*, and *CDK6*. We conclude that the exposure of cells with a chromosomal aneuploidy to TSA preferentially downregulates the expression levels of the genes located on the aneuploid chromosome and that these genes cluster together in gene signaling pathways mainly involved in cell-cycle regulation.

4 | DISCUSSION

Using gene expression profiling, we have analyzed how HDAC inhibition by TSA modulated the transcriptome in diploid and aneuploid colorectal cell lines with additional copies of chromosome 7, a genomic imbalance often observed in primary CRC.[5,6] Treatment with TSA reduced the viability of DLD-1, DLD-1 + 7, and SW480 by 30%-60%, without inducing apoptosis, and had a general stimulating effect on gene expression, as measured by increased global gene expression levels in all cell lines. All cell lines had more genes that were significantly upregulated compared to genes that were significantly downregulated after TSA treatment. The aneuploid CRC cell line SW480 showed the highest number of differentially regulated genes, while the diploid DLD-1 cells demonstrated the least differentially regulated genes. The positive effect of TSA on transcriptional activity of the whole genome has been described before in porcine mesenchymal stem cells.[17] It is believed that the transcriptional activity is mainly increased by the real-time TSA action on the direct enhancement of histone acetylation and indirect diminishment of DNA methylation.

Trisomy of chromosome 7 is one of the earliest chromosomal alterations in colorectal carcinogenesis, and this chromosomal aneuploidy is maintained during CRC progression and metastasis 18.[7] Addition of an additional copy of chromosome 7 results in low-level gene expression increases of most genes that reside on chromosome 7, although genome-wide transcriptional deregulation is observed as well.[9] As it is unknown to which extent this aneuploidy-dependent transcriptional deregulation contributes to tumorigenesis, we studied whether TSA, a compound that induces cellular differentiation and has antitumor activity in cancer cell lines, could reverse the aneuploidy-dependent gene expression changes. Previously, it has been described that despite the broad effect of TSA on the whole genome, it also has some minor site-specific action on certain chromosome regions, as it is able to selectively inhibit/stimulate gene expression via different promotors which may be connected with locus-specific acetylation patterns and chromatin structure.[17–19] Especially, genes that are highly expressed, including amplified genes, have been described to be preferentially repressed by TSA.[20] As gain of chromosome 7 is so crucial for CRC tumorigenesis, it most likely harbors important oncogenes and we expected that treatment with TSA would be able to selectively reverse overexpression of these oncogenes. Indeed, we found that TSA preferentially downregulated the gene expression on chromosome 7 in DLD-1 + 7 cells compared to DLD-1 cells. The aneuploidy-induced upregulation of chromosome 7 genes *PON2*, *ASB4*, and *ZNF273* in DLD-1 + 7 was reversed by TSA treatment. TSA treatment also resulted in

downregulation of *ASB4* in SW480. *PON2* is a member of the family of paraoxonases that localize to the endoplasmatic reticulum and to the nucleus; it has an antiapoptotic function.[21,22] *PON2* is upregulated in various tumor types, including endometrial, liver, kidney, bladder, and lymphoid cancers.[23,24] Of special interest is the fact that *PON2* expression has been described to be regulated through the Wnt/GSK3β/β-catenin pathway and that its expression was correlated with radiotherapy resistance in oral squamous cell carcinoma patients.[25] Resistance to chemoradiotherapy (CRT) occurs in 30% of the patients with rectal cancers that undergo treatment.[26] CRC carcinogenesis is associated with critical alterations in Wnt/β-catenin signaling,[27] and it has been demonstrated that preoperative CRT for locally advanced rectal cancer induced a significant increase in nuclear β-catenin expression in 49% of the patients.[28] This increase in nuclear β-catenin expression was correlated with poor survival.[28] It is already known that silencing of the Wnt pathway transcription factor *TCF7L2* results in increased sensitivity to chemoradiation of CRC cell lines.[29] Based on our study results, it would be interesting to investigate whether silencing of *PON2* could reverse CRT resistance in CRC, which might improve the prognosis of CRC patients. Also, the exact function of *ASB4* in CRC should be studied in more detail. Currently not much is known about *ASB4* with regard to its role in tumorigenesis, except for the fact that it is overexpressed in hepatocellular carcinoma (HCC) cell lines and that suppression of *ASB4* inhibited migratory and invasive properties of HCC cells.[30] *ASB4* is an especially interesting gene for future studies, as it was commonly downregulated in DLD-1 + 7 which harbors *PIK3CA* mutations and in SW480 bearing the wild-type *PIK3CA*, underlining that this gene might offer a therapeutic target in CRCs with and without *PIK3CA* mutations.[31]

Finally, we have observed that the gene-signaling pathways that were commonly downregulated by TSA treatment in both DLD-1 + 7 and SW480 cells were mainly involved in cell-cycle regulation. Genes from these significantly downregulated pathways located on chromosome 7 were *RFC2*, *PRKAR1B*, *SMO*, and *CDK6*. Inhibition of *CDK6* by PD-0332991—a selective CDK4/6 inhibitor that has been approved by the FDA for treatment of breast cancer—has already been described to induce G1 arrest in cells of several CRC cell lines, and has been suggested to be a novel therapeutic agent for treatment of CRC.[32] The inhibition of SMO protein expression has also been described to suppress proliferation of CRC cells.[33] However, the potential oncogenic roles of *RFC2* and *PRKAR1B* remain unclear, and their potential as therapeutic targets in CRC should be further investigated.

ACKNOWLEDGMENTS

The authors thank Dr Reinhard Ebner for helpful comments on the article.

ORCID

Floryne O. Buishand http://orcid.org/0000-0002-8317-1600
Thomas Ried http://orcid.org/0000-0002-0767-6009

REFERENCES

[1] Caspersson TO. Quantitative tumor cytochemistry- -G.H.A. Clowes Memorial Lecture. *Cancer Res* 1979. 39:2341–2345.

[2] Holland AJ, Cleveland DW. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol* 2009;10:478–487.

[3] Gemoll T, Roblick UJ, Szymczak S, et al. HDAC2 and TXNL1 distinguish aneuploidy from diploid colorectal cancers. *Cell Mol Life Sci* 2011;68:3261–3274.

[4] Habermann JK, Paulsen U, Roblick UJ, et al. Stage-specific alterations of the genome, transcriptome, and proteome during colorectal carcinogenesis. *Genes Chromosomes Cancer* 2007;46:10–26.

[5] Bomme L, Bardi G, Pandis N, et al. Clonal karyotypic abnormalities in colorectal adenomas: clues to the early genetic events in the adenoma-carcinoma sequence. *Genes Chromosomes Cancer* 1994;10:190–196.

[6] Ried T, Heselmeyer-Haddad K, Blegen H, et al. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. *Genes Chromosomes Cancer* 1999;25:195–204.

[7] Ried T, Knutzen R, Steinbeck R, et al. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. *Genes Chromosomes Cancer* 1996;15:234–245.

[8] Hyman E, Kauraniemi P, Hautaniemi S, et al. Impact of DNA amplification on gene expression patterns in breast cancer. *Cancer Res* 2002;62:6240–6250.

[9] Upender MB, Habermann JK, McShane LM, et al. Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res* 2004;64:6941–6949.

[10] Harada T, Chelala C, Bhakta V, et al. Genome-wide DNA copy number analysis in pancreatic cancer using high-density single nucleotide polymorphism arrays. *Oncogene* 2008;27:1951–1960.

[11] Ried T, Hu Y, Difilippantonio MJ, et al. The consequences of chromosomal aneuploidy on the transcriptome of cancer cells. *Biochim Biophys Acta* 2012;1819:784–793. (:

[12] Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. *J Clin Oncol* 2009;27:5459–5468.

[13] Inokoshi J, Katagiri M, Arima S, et al. Neuronal differentiation of neuro 2a cells by inhibitors of cell cycle progression, trichostatin A and butyrolactone I. *Biochem Biophys Res Commun* 1999;256:372–376.

[14] Radke S, Perincheri S, Schulz V, et al. The HDAC inhibitor TSA inhibits cell proliferation, induces apoptosis and down-regulates her2 protein and gene expression as a single agent and in combination with trastuzumab in trastuzumab-sensitive and -resistant breast cancer cell lines. *Cancer Res* 2009;69:690S.

[15] Fortson WS, Kayarthodi S, Fujimura Y, et al. Histone deacetylase inhibitors, valproic acid and trichostatin-A induce apoptosis and affect acetylation status of p53 in ERG- positive prostate cancer cells. *Int J Oncol* 2011;39:111–119.

[16] Xiong H, Du W, Zhang YJ, et al. Trichostatin A, a histone deacetylase inhibitor, suppresses JAK2/STAT3 signaling via inducing the promoter-associated histone acetylation of SOCS1 and SOCS3 in human colorectal cancer cells. *Mol Carcinog* 2012;51:174–184.

[17] Gurgul A, Opiela J, Pawlina K, et al. The effect of histone deacetylase inhibitor trichostatin A on porcine mesenchymal stem cell transciptome. *Biochemie* 2017;139:56–73.

[18] Takahashi I, Miyaji H, Yoshida T, et al. Selective inhibition of IL-2 gene expression by trichostatin A, a potent inhibitor of mammalian histone deacetylase. *J Antibiot* 1996;49:453–457.

[19] Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylase inhibitors: from bacteria and yeast to mice and men. *Expert Opin Ther Pat* 2008;19:1727–1757.

[20] Kim YJ, Greer CB, Cecchini KR, et al. HDAC inhibitors induce transcriptional repression of high copy number genes in breast cancer through elongation blockade. *Oncogene* 2013;32:2828–2835.

[21] Draganov DI, Teiber JF, Speelman A, et al. Human paraoxonases (pon1, pon2, and pon3) are lactonases with overlapping and distinct substrate specificities. *J Lipid Res* 2005;46:1239–1247.

[22] Horke S, Witte I, Wilgenbus P, et al. Paraoxonase-2 reduces oxidative stress in vascular cells and decreases endoplasmic reticulum stress-induced caspase activation. *Circulation* 2007;115:2055–2064.

[23] Witte I, Altenhofer S, Wilgenbus P, et al. Beyond reduction of atherosclerosis: Pon2 provides apoptosis resistance and stabilizes tumor cells. *Cell Death Dis* 2011;2:e112.

[24] Witte I, Foerstermann U, Devarajan A, et al. Protectors or traitors: The roles of pon2 and pon3 in atherosclerosis and cancer. *J Lipids* 2012;2012:342806.

[25] Krüger M, Amort J, Wilgenbus P, et al. The anti-apoptotic PON2 protein is Wnt/β-catenin-regulated and correlates with radiotherapy resistance in OSCC patients. *Oncotarget* 2016;7:51082–51095.

[26] Sauer R, Liersch T, Merkel S, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. *J Clin Oncol* 2012;30:1926–1933.

[27] Clevers H. Wnt/β-catenin signaling in development and disease. *Cell* 2006;127:469–480.

[28] Gomez-Millan J, Perez L, Aroca I, et al. Preoperative chemoradiotherapy in rectal cancer induces changes in the expression of nuclear β-catenin: prognostic significance. *BMC Cancer* 2014;14:192.

[29] Kendziorra E, Ahlborn K, Spitzner M, et al. Silencing of the Wnt transcription factor TCF4 sensitizes colorectal cancer cells to (chemo-) radiotherapy. *Carcinogenesis* 2011;32:1824–1831.

[30] Au V, Tsang FH, Man K, et al. Expression of Ankyrin repeat and SOCS box containing 4 (ASB4) confers migration and invasion properties of hepatocellular carcinoma cells. *Biosci Trends* 2014;8:101–110.

[31] Ahmed D, Eide PW, Eilertsen IA, et al. Epigenetic and genetic features of 24 colon cancer cell lines. *Oncogenesis* 2013;2:e71.

[32] Li C, Qi L, Bellail AC, et al. PD-0332991 induces G1 arrest of colorectal carcinoma cells through inhibition of the cyclin-dependent kinase-6 and retinoblastoma protein axis. *Oncol Lett* 2014;7:1673–1678.

[33] Sun K, Deng HJ, Lei ST, et al. miRNA-338-3p suppresses cell growth of human colorectal carcinoma by targeting smoothened. *World J Gastroenterol* 2013;19:2197–2207.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Buishand FO, Cardin E, Hu Y, Ried T. Trichostatin A preferentially reverses the upregulation of gene-expression levels induced by gain of chromosome 7 in colorectal cancer cell lines. *Genes Chromosomes Cancer*. 2018;57:35–41. https://doi.org/10.1002/gcc.22505