Genomics Data 7 (2016) 293–296

Contents lists available at ScienceDirect

Genomics Data

journal homepage: www.elsevier.com/locate/gdata

Differential RNA-seq analysis comparing APC-defective and APC-restored SW480 colorectal cancer cells

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 31 January 2016
Accepted 1 February 2016
Available online 3 February 2016

Keywords:
Adenomatous polyposis coli
Wnt signalling
Colorectal cancer
RNA-seq
Gene expression

ABSTRACT

The *adenomatous polyposis coli* (*APC*) tumour suppressor gene is mutated in about 80% of colorectal cancers (CRC) Brannon et al. (2014) [1]. APC is a large multifunctional protein that regulates many biological functions including Wnt signalling (through the regulation of beta-catenin stability) Reya and Clevers (2005) [2], cell migration Kroboth et al. (2007), Sansom et al. (2004) [3,4], mitosis Kaplan et al. (2001) [5], cell adhesion Faux et al. (2004), Carothers et al. (2001) [6,7] and differentiation Sansom et al. (2004) [4]. Although the role of APC in CRC is often described as the deregulation of Wnt signalling, its other biological functions suggest that there are other factors at play that contribute to the onset of adenomas and the progression of CRC upon the truncation of APC. To identify genes and pathways that are dysregulated as a consequence of loss of function of APC, we compared the gene expression profiles of the *APC* mutated human CRC cell line SW480 following reintroduction of wild-type APC (SW480 + APC) or empty control vector (SW480 + vector control) Faux et al. (2004) . Here we describe the RNA-seq data derived for three biological replicates of parental SW480, SW480 + vector control and SW480 + APC cells, and present the bioinformatics pipeline used to test for differential gene expression and pathway enrichment analysis. A total of 1735 genes showed significant differential expression when APC was restored and were enriched for genes associated with cell polarity, Wnt signalling and the epithelial to mesenchymal transition. There was additional enrichment for genes involved in cell–cell adhesion, cell–matrix junctions, angiogenesis, axon morphogenesis and cell movement. The raw and analysed RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE76307. This dataset is useful for further investigations of the impact of *APC* mutation on the properties of colorectal cancer cells.

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Specification	
Organism/cell line/tissue	SW480 Human epithelial cell line
Sex	Male
Sequencer or array type	Illumina HiSeq RNASEQ
Data format	Raw and analysed
Experimental factors	Colorectal cancer cell line with/without functional APC
Experimental features	Determine gene expression alterations in the presence/ absence of APC gene in the colorectal cancer context.
Consent	NA
Sample source location	Melbourne, Australia

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[1] These authors contributed equally to this study.

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76307.

2. Experimental design, material and methods

2.1. Cell culture and RNA-extraction

The SW480, SW480 + APC (SW480APC.15) and SW480 + control (SW480control.7) cells have been previously described [6]. The cells were thawed from liquid nitrogen and grown in RPMI supplemented with 0.001% thioglycerol, 1 μg/ml hydrocortisone, 0.025 U/ml insulin, 10% foetal calf serum and 1% penicillin/streptomycin (SW480 cells)

http://dx.doi.org/10.1016/j.gdata.2016.02.001
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Table 1
Summary sequencing statistics for RNA-seq data.

Sample	Replicate	Raw reads	Trimmed reads	Aligned reads
SW480 + APC.1	1	22,518,756	21,966,108	21,050,021
SW480 + APC.2	2	21,381,045	20,806,066	19,682,638
SW480 + APC.3	3	20,825,899	20,295,588	19,438,311
SW480 + Control.1	1	22,596,671	22,056,529	21,039,650
SW480 + Control.2	2	19,832,005	19,354,879	18,551,827
SW480 + Control.3	3	23,352,479	22,793,011	21,855,257
SW480.1	1	22,075,530	21,528,124	20,699,835
SW480.2	2	22,389,667	21,835,786	20,798,952
SW480.3	3	22,563,469	22,007,875	21,000,302

plus 1.5 mg/ml G418 (SW480 + APC and SW480 + control cells). Cells were passaged three times before being plated onto 100 mm tissue culture plates at a density of 3.35×10^5 for SW480 and SW480 + control cells and 2×10^5 for SW480 + APC cells, in triplicate. Seventy two hours later RNA was extracted from the cells using the RNAspin Mini RNA isolation kit (Illustra 25-0500-70).

2.2. Sequencing, mapping and normalisation

RNA samples were prepared for sequencing using the Illumina TruSeq RNA Library Preparation Kit v2. Libraries were pooled (9 samples per pool) and clustered using the Illumina cBot system with TruSeq SR Cluster Kit v3 reagents, followed by sequencing on the Illumina HiSeq 2000 system with TruSeq SBS Kit v3 reagents (101 cycles) at the Australian Genome Research Facility. Each sample was sequenced to a depth of approximately 20 million reads (see Table 1). Sequencing reads were quality assessed and trimmed for any remaining sequencing adaptor using Trimmomatic (v0.22) [8]; reads smaller than 50 bp were removed. Reads were subsequently aligned to human genome build Hg19 using Tophat (v2.0.6.Linux_x86_64) [9] with parameters -g 1

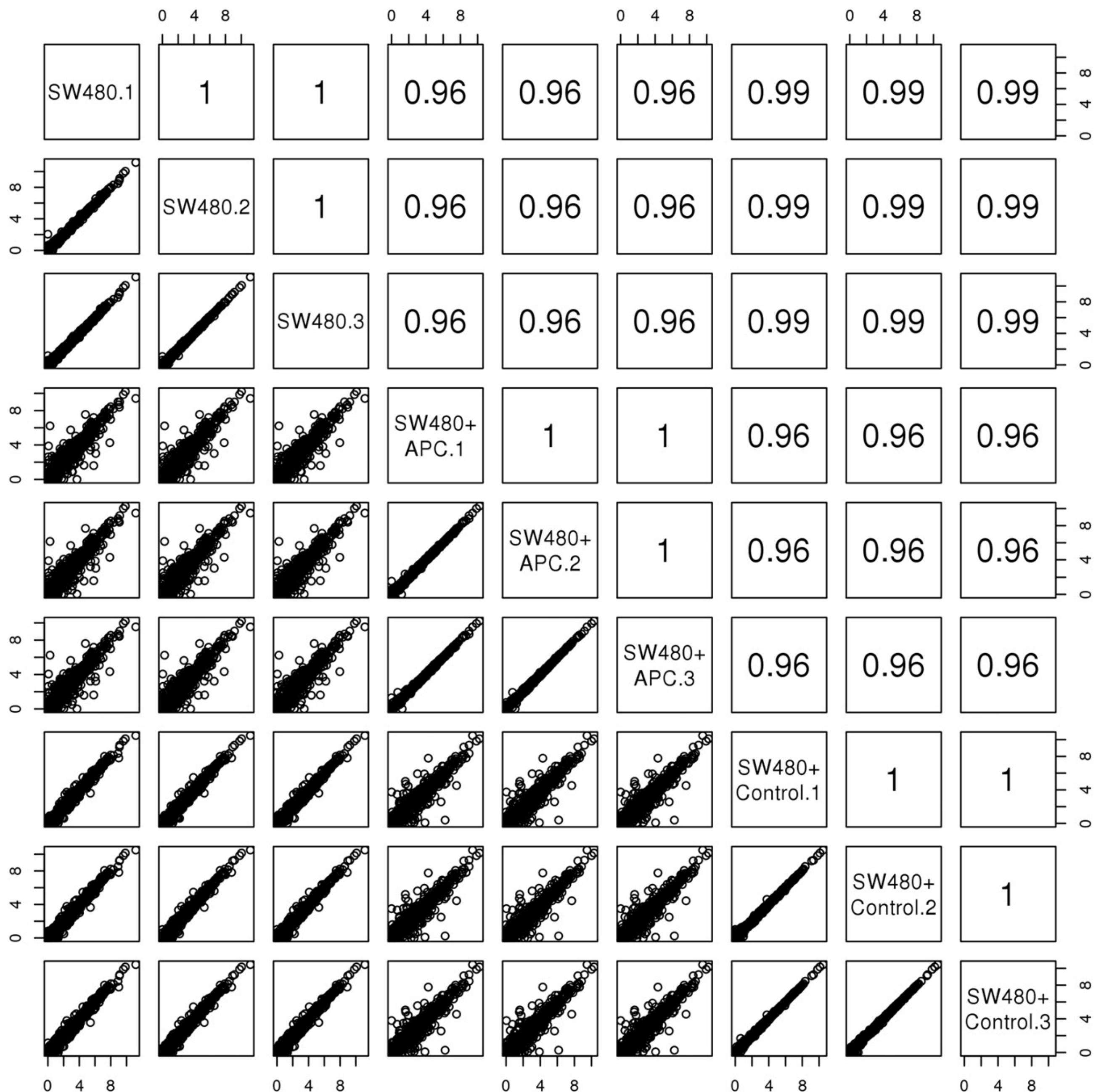

Fig. 1. Scatter plots of log2 expression values (RPKM) for 1000 randomly selected genes between cell line samples. Pearson correlation coefficients are indicated in the top half of quadrant.

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