Coronary Artery Disease Associated Transcription Factor TCF21 Regulates Smooth Muscle Precursor Cells that Contribute to the Fibrous Cap

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Abstract

TCF21 is a basic helix-loop-helix transcription factor that has recently been implicated as contributing to susceptibility to coronary heart disease based on genome wide association studies. In order to identify transcriptionally regulated target genes in a major disease relevant cell type, we performed siRNA knockdown of TCF21 in in vitro cultured human coronary artery smooth muscle cells and compared the transcriptome of siTCF21 versus siCONTROL treated cells. The raw (FASTQ) as well as processed (BED) data from 3 technical replicates per treatment has been deposited with Gene Expression Omnibus (GSE44461)

Specifications

Organism/cell line/tissue	*Human Coronary Artery Smooth Muscle Cells(HCASMC)*
Sex	*Male/female*
Sequencer or array type	*Illumina HiSeq2000*
Data format	*Raw and analyzed*

*Equal contribution

Experimental factors	*siRNA treatment: siTCF21; siCONTROL*
Experimental features	*HCASMCs were purchased from Lonza and cultured according to the manufacturer's recommendations. Cells were serum-starved and transfected with siRNA pools from OriGene. Total RNA was collected and RiboZero libraries generated and sequenced on an Illumina HiSeq2000 instrument. The resulting reads were mapped to hg19 with TopHat(1)/Bowtie2(2). Differentially expressed genes were identified using either DESeq (3) and edgeR(4).*
Consent	*manufacturer's informed donor consent*
Sample source location	*n/a*

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44461

Experimental Design, Materials and Methods

Cell culture

Human primary Coronary Artery Smooth Muscle Cells (HCASMC, Lonza #CC-2583 Lot 200212 and Cell Applications # 350-05a Lot 1508) were cultured in Smooth Muscle Growth Medium-2 including hEGF, insulin, hFGF-B and FBS, but without antibiotics (Lonza, #CC-3182). For RNA-Seq studies donor-pooled HCASMC were transfected with 300 nM TCF21 Trilencer-27 Human siRNA (OriGene #SR304753C) or Trilencer-27 Universal Scrambled Negative Control siRNA (OriGene #SR30004) at 80% confluence using the Amaxa Basic Nucleofector Kit for Primary Mammalian Smooth Muscle Cells (Lonza #VPI-1004) at a density of 1×106 cells per 100 μL sample using Nucleofector Program U-025. Cells were changed to medium with supplements at 18 hours post-transfection and cultured for an additional 48 hours.

RNA sequencing

Total RNA from either siTCF21 or siCTRL treated samples was depleted for ribosomal RNA with the Ribo-Zero magnetic kit from Epicentre (Illumina #MRZH116), libraries generated with the Epicentre ScriptSeq v2 RNA-Seq library preparation kit (Illumina #SSV21106) and thereafter sequenced as 100bp paired-end reads on an Illumina HiSeq 2000 instrument. The resulting data has been deposited at GEO under accession number GSE44461. Reads resulting from RNA-Sequencing of siCTRL and siTCF21 treated HCASMC were mapped using software tools TopHat+Bowtie2. Differential expression level between samples was analyzed using the software tools DESeq and edgeR at an FDR 0.05, with intersection of the 466 and 430 respective identified genes providing a group of 380 common genes.

References

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