Discovery of a role for the single START-domain protein Mtsp17 in transcriptional regulation

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Abstract

Background

Tuberculosis caused by the pathogen *Mycobacterium tuberculosis* (MTB), remains a significant threat to global health. Elucidating the mechanism of essential MTB genes will provide a theoretical basis for drug exploitation. Gene *mtsp17* is essential and conserved in the Mycobacterium genus. Although the structure of Mtsp17 exhibits as a typical steroidogenic acute regulatory protein-related lipid transfer (START) family protein, its biological function is different from other START proteins. This study characterized the transcriptomes of *Mycobacterium smegmatis* to address the problem how suppressing *mtsp17* reduces bacterial growth rate.

Results

3% down- and 1% up-regulated protein-coding genes were significantly differentiated expressed by suppressing *mtsp17* gene. Among them, *desA1*, an essential gene involved in mycolic acid synthesis, was found to be significantly depressed (3.1-fold). Further analyses showed that *mstp17* can activate *desA1* to regulate the bacterial growth. In addition, the anti-anti-SigF gene was dramatically decreased (8.9-fold) and 70 of the 79 differentially expressed genes showed the same change trends in the SigF knockout strain. The data indicate that Mtsp17 regulates SigF regulon by modulating mRNA abundance of the anti-sigma factor antagonist.

Conclusions

Our findings discover the role of Mtsp17 in transcriptional regulation that was unknown before, which provides new insights into the molecular mechanisms of START family and introduces a new node to the regulatory network of mycobacteria.

Background

A 17-kDa protein isolated from MTB culture filtrate was identified as a T- and B-cell antigen and named Mtsp17 [1, 2]. Mtsp17 is encoded by Rv0164 gene in MTB and is highly conserved in Mycobacterium species. There is no homologous sequence in other genus that shares more than 30% identity with MTB Mtsp17, thus Mtsp17 can be not only an anti-TB drug target but also a candidate immunogen for vaccine development. Although *mtsp17* has been demonstrated as an essential gene in both *Mycobacterium smegmatis* (MSM) and MTB [3, 4], its physiological function is presently unclear. Considering its essentiality and exclusiveness in the genus Mycobacterium, Mtsp17 needs more investigation which will introduce new insights into understanding the physiological properties of mycobacteria.

Mtsp17 folds as polyketide cyclases/aromatases (CYC/ARO) although they share sequence identity lower than 20% [3]. CYC/ARO are monofunctional enzymes in the Type II polyketide synthase (PKS) complexes and transform linear poly-*β*-ketone intermediates into aromatic polyketides [5]. But no Type II PKS has yet been identified in mycobacteria while 21 Type I PKSs and three Type III have been annotated [6]. Structural comparisons and docking infer that Mtsp17 is likely not enzyme that catalyzes polyketide cyclization and aromatization [3]. Therefore Mtsp17 may play essential roles in mycobacteria by a mechanism different from CYC/ARO.

Mtsp17 is a single-domain protein that has the steroidogenic acute regulatory protein-related lipid transfer (START) domain. START are lipid-binding domains working as polyketide cyclases/aromatases involved in lipid metabolism. The START domain also can bind ligand in non-vesicular traffic between intracellular compartments and fuse with DNA binding domain to modulate gene transcription [7–9]. As mycobacteria lacks inter-organelles such as endoplasmic reticulum and Golgi apparatus, it is speculated that START domain in Mtsp17 may regulate transcription through a DNA contact-independent mechanism. A comprehensive profiling of the genes and pathways regulated by Mtsp17 will provide insights into its physiological functions.

As *mtsp17* knockout is lethal to MSM, a *mtsp17* complemented strain (M0129C) was previously generated in which plasmid-encoded Mtsp17 is induced by tetracycline while the genomic *mtsp17* gene is deleted [3]. In this study, RNA-sequencing (RNA-seq) based transcriptomics analysis was performed to obtain a global profile of genes regulated by Mtsp17 in MSM. We compared the transcriptome profiles between wildtype and the *mtsp17* complemented strains (*mtsp17* induced and not induced) and discovered transcriptional regulatory properties of Mtsp17. The transcriptional regulatory mechanism is discussed.

Results

Differentially expressed genes varied dramatically among the WT and complemented strains

The sample M0129C_T20 was induced with tetracycline while M0129C_T0 was not. *M. smegmatis* mc^2155 was applied as a control (Wt). To identify differentially expressed genes (DEGs), pair-wise comparisons of read counts between the complemented strain and wildtype were performed using DESeq (Supplementary Table 2). Although *mtsp17* was down regulated in the 3 groups (M0129C_T0 versus T20; Wt versus M0129C_T20; Wt versus M0129C_T0), DEGs varied dramatically in quantity. Overall 4% protein-coding genes were DEGs (fold change > 2 and adjusted p-value < 0.05) in the M0129C_T0 versus T20 group while 24–25% in other 2 groups (Table 1). 50 DEGs (fold change > 2) overlapped between the three groups (Fig. 1A, Supplementary Table 3), among which 24 genes (including *mtsp17*) showed the same trends of fold change and 7 of these 24 genes have homolog in MTB (Fig. 1B, Table 2). The same change trends of DEGs among the 3 groups indicate that Mtsp17 has an important role of gene expression

regulation. Furthermore, 15 genes were up regulated while 8 of the 23 DEGs were down regulated, implying that Mtsp17 could act as both an activator and a repressor.

Difference between the expression patterns of complemented strains was more moderate

Principal component analysis of the global expression patterns of the 3 samples demonstrated that wildtype clustered apart from the two complemented strain samples, and M0129C_T0 clustered apart from M0129C_T20 with the majority of variance occurring along PC2 (Fig. 1C). In addition, hierarchical clustering with the DEGs (adjusted p-value < 0.05) showed that gene expression pattern of the M0129C_T20 sample was more similar to M0129C_T0 (Fig. 1D). These results indicate that the difference between the expression patterns of M0129C_T20 and M0129C_T0 is more moderate than between the complemented strain and wildtype. Therefore focusing on the DEGs of the M0129C_T0 versus T20 group is more apt to unveil the mechanism that regulates the gene expression profile by Mtsp17 depression.

Table 1
Numbers of genes differentially expressed

Group		M0129C_T0 /T20	Wt/ M0129C_T20	Wt/ M0129C_T0
mtsp17 ratio (log_2 FC)		-2.4	-5.4	-3
Differentially expressed genes (Padj < 0.05, log_2 FC)	Down > 1	3% (207)	11% (797)	12%(861)
	Up > 1	1% (68)	13%(896)	13%(916)
	FC < 1	20%(1370)	17% (1150)	18%(1273)
Others		76%	59%	57%

Data were from RNA-seq with 3 replicates of each sample. FC, fold change. Padj, adjusted p-value.

Table 2
Partial DEGs overlapped between the pair-wise comparisons

MSM	MTB	Essential[4]	Description	Functional category
MSMEG_0129	Rv0164	Yes	Mtsp17	Unknown
MSMEG_3763	Rv1686c	No	ABC transporter	Cell wall and cell processes
MSMEG_0787	Rv0411c	No	extracellular solute-binding protein	Cell wall and cell processes
MSMEG_0788	Rv0412c	No	hypothetical protein	Cell wall and cell processes
MSMEG_2850	Rv3178	No	cell entry related family protein	Unknown
MSMEG_1769	Rv3288c	No	UsfY protein	Unknown
MSMEG_4757	Rv2524c	Yes	fatty acid synthase	Lipid metabolism

Qpcr Analyses Verified The Rna Sequencing Data

Down regulation of Mtsp17 resulted in induction of 808 genes and repression of 837 genes (adjusted p-value < 0.05), representing 1645 transcriptional targets that related to Mtsp17 (Fig. 2A). 13 DEGs were randomly selected for qPCR analyses to verify the gene expression changes observed via RNA sequencing. The trend in fold changes of qPCR data matched RNA-seq results (Fig. 2B), thereby validating the down-regulated Mtsp17 transcriptome profile generated by RNA sequencing.

Mtsp17 acted as a transcriptional activator of desA1 **gene**

Gene ontology (GO) terms of the DEGs were assessed by GOseq v1.22 and 7 GO terms were significantly enriched (adjusted p-value < 0.05), including “structural constituent of ribosome” (GO:0003735) and “ribonucleoprotein complex” (GO:0030529) (Fig. 2C). In addition, quantity information of 58 ribosomal proteins was obtained in the RNA-seq data and 27 were DEGs (adjusted p-value < 0.05). But fold changes of all the 27 differentially expressed ribosomal proteins (twelve 30S ribosomal proteins and fifteen 50S ribosomal proteins) were less than 2.

Four of the 275 significantly differentially expressed genes (adjusted p-value < 0.05 and fold change > 2, Supplementary Table 4) were essential for mycobacterial growth, but only *desA1* (MSMEG_5773) was down-regulated by depression of Mtsp17. DesA1 is a desaturase involved in mycolic acid biosynthesis in mycobacteria [10]. Changes of mycolic acids are central to adaptation of MTB to various environments [11, 12]. Transcription of MTB *desA1* is regulated by the transcription factor MadR while overexpression of other transcription factors leads no change of *desA1* mRNA [13]. The MSM MadR (encoded by MSMEG_0916) and DesA1 (encoded by MSMEG_5773) proteins show high similarity to the homologs in

MTB (85% and 80% identity), respectively. The evolutionarily conserved MadR-mediated *desA1* repression leads to decrease of mycolic acid biosynthesis and ensues loss of mycobacterial viability [12]. Analogously, the decline of bacterial growth in insufficient Mstp17 strains is likely due to depression of *desA1* by down regulating *mtsp17*.

More transcriptome data related to Mtsp17-*desA1* regulation were searched and summarized in Table 3. MadR overexpression made no significant change of *mtsp17* mRNA in both MTB and MSM. The same phenotype was also found in MTB when 206 transcription factors were overexpressed respectively [13]. These phenomena indicate that Mtsp17 is probably an upstream factor of transcription factors which supports our hypothesis that Mtsp17 may regulate transcription through a DNA contact-independent mechanism. Under hypoxia, transcriptional change patterns of *madR* and *desA1* were not in accordance with MadR repressing *desA1* and could be explained by Mtsp17 activating *desA1* (Table 3). The above results indicate that Mtsp17 plays as a transcriptional activator of *desA1* gene.

Table 3
Log_2 fold change value of genes in different pair-wise comparisons

Genes	MadR overexpression	MadR overexpression	Mtsp17 downregulation	Hypoxia
madR	1.48	1.82	0.13	-0.16
desA1	-1.46	-1.75	-1.62	-3.25
mtsp17	-0.18	0.06	-2.41	-1.53
Species	MSM	MTB	MSM	MTB
GEO*	GSE116027	GSE59086	This study	GSE116353
*the Gene Expression Omnibus (GEO) series accession number.				

Characterization of SigF regulon in the mtsp17 **complemented strain**

Transcription in MSM is carried out by a multi-subunit RNA polymerase and one of the 28 sigma subunits [14]. The sigma subunit ensures the transcription machinery to initiate transcription of particular genes. Sigma factor is post-translationally regulated by its cognate anti-sigma factors, which sequester them from the transcription machinery and could be neutralized by anti-sigma factor antagonists [15].

There was one anti-sigma factor or its antagonist, which is an anti-sigma factor antagonist of SigF (MSMEG_0586, 8.9-fold decrease, adjusted p-value = 7.3E-40) among the top 20 differentially expressed genes in the M0129C_T0 versus M0129C_T20 group. SigF controls the expression of a particular subset of genes (SigF regulon) by altering RNA polymerase specificity [16]. The DEGs in the M0129C_T0 versus M0129C_T20 group (M0129C_T0/T20) were compared with fold changes in a SigF mutant [17]. 70 of seventy nine DEGs with log_2 fold change more than 1.5 and adjusted p-value less than 0.05 showed the same trend of fold changes (Supplementary Table 5). Furthermore, 10 of the seventy nine DEGs are

involved in transcriptional regulatory mechanism and they all showed the same trend in SigF knockout versus wildtype group and M0129C_T0 versus M0129C_T20 group except MSMEG_0529 (Fig. 3A). The above results indicate that the majority of significantly differentially expressed genes regulated by Mtsp17 belong to SigF regulon.

MSMEG_0529 encodes the Serine/threonine-protein kinase PknK and participates in regulatory pathways that slow growth of *M. tuberculosis* under stresses [18]. SigF overexpression produced moderate upregulation of *pknK* ($\log_2$ fold change = 0.91) [13]. Here, down regulation of *mtsp17* led to decrease of the anti-SigF antagonist gene (8.9-fold) and upregulation of *pknK* (2.9-fold). These results indicate that Mtsp17 rather than SigF is the key factor of the *pknK* regulatory network. More details are needed to be explored to full fill the regulatory relationships between *pknK* gene and Mtsp17.

Discussion

Essential MTB genes are good targets for drug discovery [19]. Elucidating the mechanism of essential genes will be benefit to the development of more effective vaccines and better drugs. Mtsp17 has many special features, including: (1) no homologs in other genus; (2) essentiality for mycobacterial growth; (3) no such enzymatic activity as other bacterial START family proteins. Here we illustrate that Mtsp17 is involved in gene regulatory networks and demonstrate for the first time that START family protein with mono domain could regulate transcription in MSM.

Initiation of transcription by RNA polymerase (RNAP) has been proved to be different between mycobacteria and *Escherichia coli* (*E. coli*), and mycobacterial RNAP forms a more unstable RNAP-promoter open complex (RPo) [20]. Therefore, special transcription regulators are needed to support mycobacterial growth and gene regulatory networks are very complicated. For example, the transcription factors CarD and RbpA which are absent in *E. coli* perform essential roles for mycobacterial growth by stabilizing RPo [21, 22], and interaction between RbpA and SigB regulates PPK1 transcription in mycobacteria [23]. The specificity of Mtsp17 probably adapts to the unstable mycobacterial RPo. Considering that Mtsp17 is a lipid-binding protein and lack of DNA-binding domain, this transcriptional regulator may work in a DNA-contact independent way by protein-protein interactions. Such mechanism has been utilized by Crl in *E. coli*, which activates transcription by interacting with sigma factor [24]. More investigations are needed to explore the potential interacting protein of Mtsp17 to complete the transcriptional regulatory network of Mtsp17.

Conclusions

In this study, the consequences of down-regulating Mtsp17 were characterized by RNA sequencing. The essential gene *desA1* was found to be significantly depressed (3.1 fold) in the M0129C_T0 versus T20 group, in accordance with the phenotype that down-regulation of Mtsp17 reduced bacterial growth. Further analyses implied that Mstp17 is an activator of *desA1*. Additionally, anti-anti-SigF was also dramatically decreased and 88% (70/79) differentially expressed genes of M0129C_T0 versus T20 group

showed the same change tendency in the SigF knockout versus wildtype group, indicating Mtsp17 regulates the SigF regulon. Therefore, it is concluded that Mtsp17 is involved in transcriptional regulation by the patterns of 1) SigF dependent mechanism and 2) SigF-independent mechanism which targets the essential gene *desA1* (Fig. 3B).

Methods

Bacterial strains and culture conditions

Mycobacterium smegmatis strains were cultured in Middlebrook 7H9 medium (Difco, Baltimore, MD, USA) supplemented with 0.5% glycerol and 0.05% Tween 80 at 37°C. 25 $\mu g \cdot mL^{-1}$ kanamycin and 20 $ng \cdot mL^{-1}$ tetracycline (Sangon Biotech, Shanghai, China) was required for culturing the *mtsp17* complemented strain in which genomic *mtsp17* gene was replaced by the sacB-hyg cassette and extrachromosomal *mtsp17* gene was introduced by a pMind derivative plasmid. When the cultures of complemented strain reached exponential phase in the presence of tetracycline (OD_{600} = 0.4), they were transferred into fresh culture medium and divided into two halves. One half was added with tetracycline 20 $ng \cdot mL^{-1}$ (M0129C_T20, induced) while the other without tetracycline (M0129C_T0, not induced) during 4 more hours incubation. Then the two cultures of the complemented strain were collected for total RNA isolation and sequencing. Exponential phase culture of *Mycobacterium smegmatis* mc^2155 was applied as a control (Wt).

RNA isolation and transcriptome analysis

10 ml of exponentially growing cultures were harvested by centrifugation (4 °C, 3000 rpm, 10 min). Total RNA was isolated from the pellet using the FastPrep Instrument and FastRNA Pro Blue Kit (MP Biomedicals, Solon, OH, USA) according to the application manual provided by the manufacturer. Then RNA was treated with DNase I and precipitated with ammonium acetate/isopropanol. Quality and quantity of each RNA sample were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Beijing, China) and a NanoDrop 1000 spectrophotometer (Thermofisher, Shanghai, China). The Ribo-Zero rRNA Removal Kit (Epicentre, Madison, WI, USA) was used to remove bacterial rRNA from total RNA preparations.

The cDNA libraries were constructed using NEBNextUltraTM RNA library Prep Kit (New England Biolabs, Beijing, China) and sequenced on a HiSeq 4000 sequencer (Illumina). The processed reads were mapped to the *Mycobacterium smegmatis* MC^2 155 genome (NC_008596) using Bowtie2 v2.2.6 with default parameters. FPKM values for each gene and differentially expressed genes (DEGs) were analyzed with HTSeq v0.6.0 and DESeq v1.22.1. Genes with multiple hypothesis-adjusted p-value below 0.05 (adjusted p-value < 0.05) were considered DEGs. Functional enrichment analysis was carried out by GOseq v1.22 based on Gene Ontology database.

Real-time fluorescence quantification PCR (qPCR) analysis

Selected genes were measured by qPCR in a CFX96 instrument (Bio-Rad). GoScript Reverse Transcription System (Promega, Beijing, China) was used for cDNA preparation. 2x SYBR Green mix (Vazyme, Nanjing, China) and gene-specific primers (Supplementary Table 1) were used for amplification (95°C, 10 s; 60°C, 30 s; 40 cycles). Primers were optimized to be 90-110% amplification efficiency and single melting temperature. *sigA* (MSMEG_2758) was used as an internal normalization standard. Relative quantification was carried out by the ΔΔCT method.

Abbreviations

START, steroidogenic acute regulatory protein-related lipid transfer; qPCR, Real-time fluorescence quantification PCR; MTB, Mycobacterium tuberculosis; MSM, Mycobacterium smegmatis; E. coli, Escherichia coli; CYC/ARO, cyclases / aromatases; PKS, polyketide synthase; RNA-seq, RNA-sequencing; RNAP, RNA polymerase; RPo, RNA polymerase-promoter open complex; GO, gene ontology; DEGs, differentially expressed genes.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Ying Zhou, Wenjing Wei, Zhuhua Wu and Anping Yang did RNA isolation and RNA sequencing. Ying Zhou and Ming Wang analyzed data. Ying Zhou, Tianying Zhong and Xiaoli Zhang wrote the manuscript.

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Figures

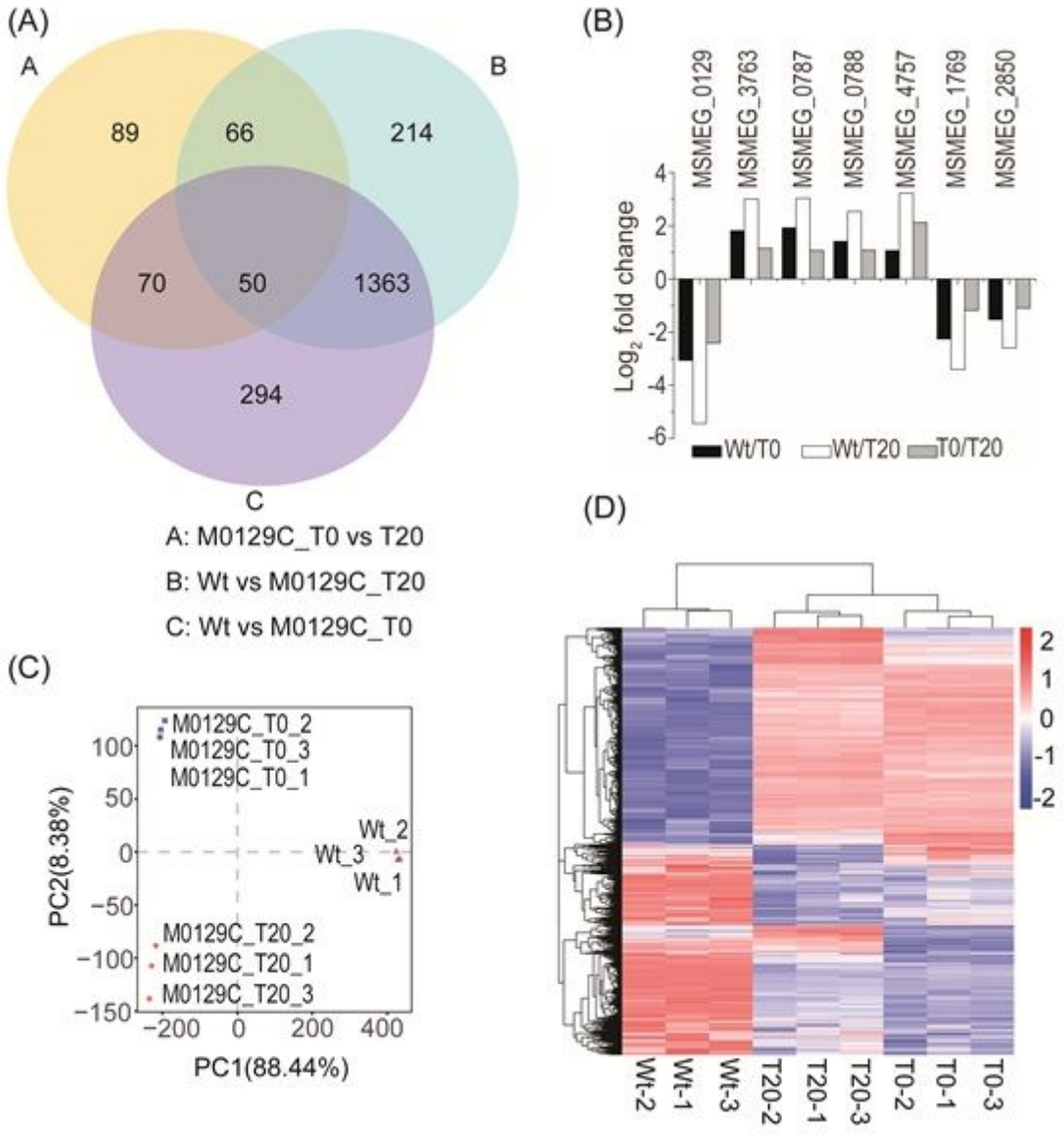

Figure 1

Differentially-expressed genes in the pair-wise comparisons. (A) Venn diagram showing the numbers of significantly differentially expressed genes (fold change > 2, adjusted p-value < 0.05). (B) Differentially-expressed genes showing the same trends of fold changes. (C) Principal component analysis of the global expression patterns of the 3 samples. (D) Hierarchical clustering with the 2146 DEGs (adjusted p-value < 0.05).

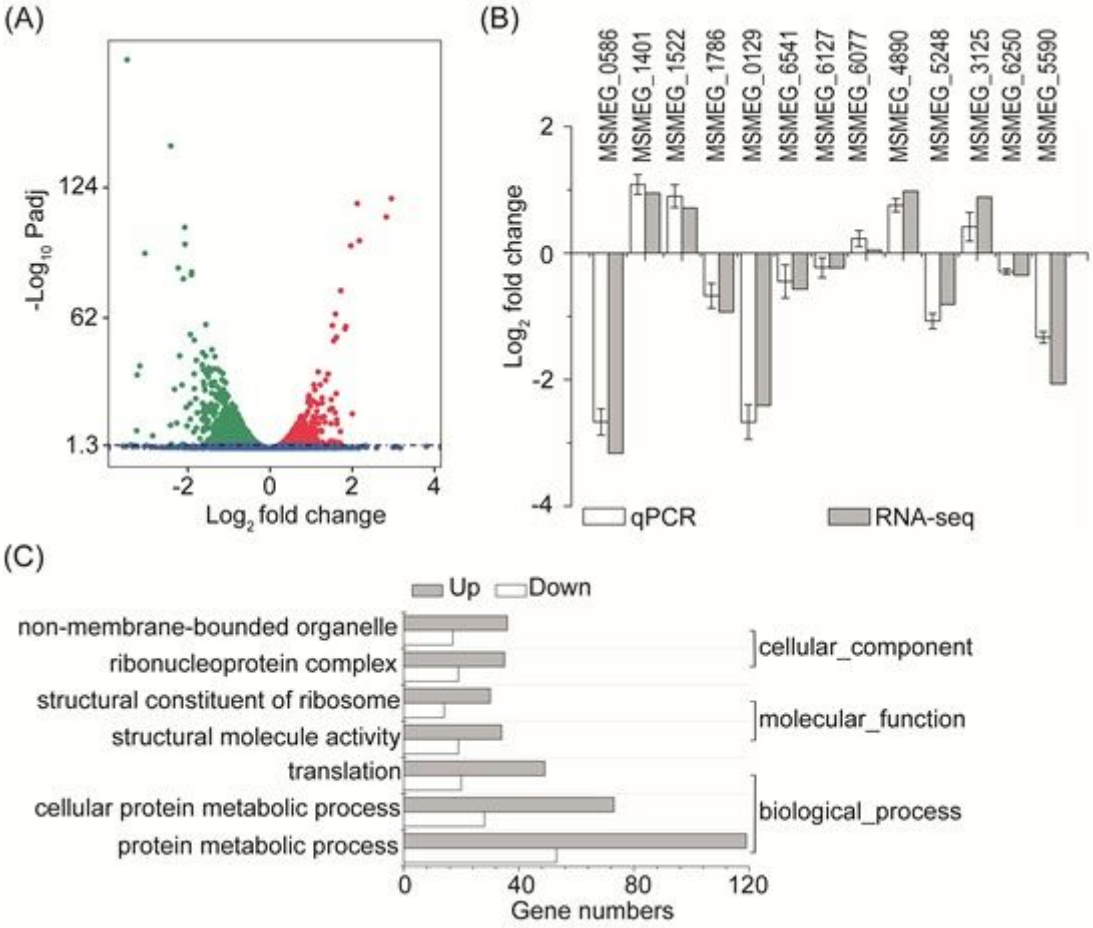

Figure 2

Differentially-expressed genes in the M0129C_T0 versus M0129C_T20 group. (A) Volcano plot showing differentially expressed genes. Green, down-regulated genes. Red, up-regulated genes. Blue, not differentially expressed genes. Padj, adjusted p-value. (B) qPCR analyses of 13 randomly selected DEGs. (C) Significantly enriched gene ontology (GO) terms (adjusted p-value < 0.05).

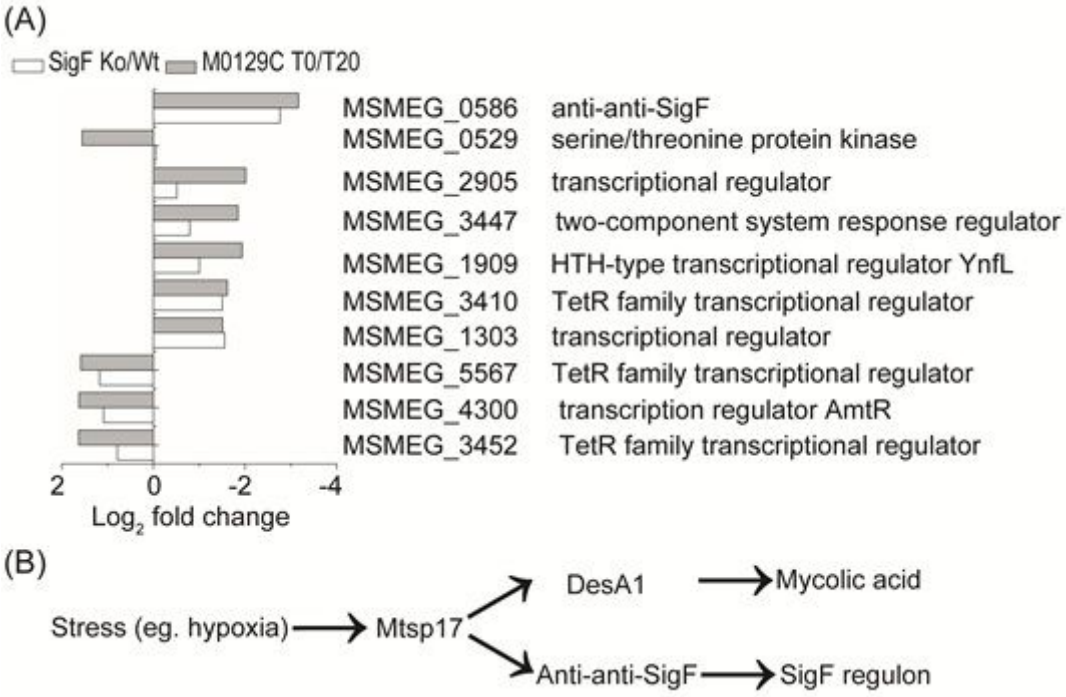

Figure 3

Partial differentially-expressed SigF regulon genes. (A) Fold change value of differentially expressed genes involved in transcription regulation. Data of SigF knockout versus wildtype group was from [17]. (B) Model of Mtsp17 involved in transcriptional regulation.

Supplementary Files

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- SupplementaryTable15.xlsx