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Effects of curcumin on the parasite *Schistosoma mansoni*: A transcriptomic approach

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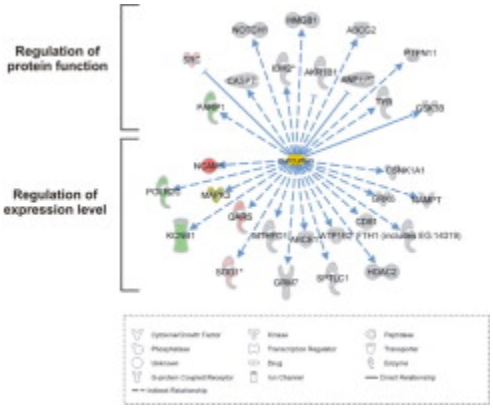
Abstract

Schistosomiasis remains a severe problem of public health in developing countries. Several reports show that praziquantel, the drug of choice for treating schistosomiasis, can select *Schistosoma mansoni* strains resistant to the drug. Thus, developing new drugs against this parasitosis is a highly desirable goal. Curcumin, a phenolic compound deriving from the plant *Curcuma longa*, has been shown to have anticancer, anti-inflammatory and antiparasitic effects. Recently, our group has demonstrated that curcumin causes the separation of *S. mansoni* adult worm pairs, eggs infertility, decreased oviposition and parasite viability, leading to death. In the present work, we have investigated the effects of curcumin on *S. mansoni* gene expression in adult worms through microarray analyses. Our results showed 2374 genes that were significantly and differentially expressed, of which 981 were up-regulated and 1393 were down-regulated. Among the differentially expressed genes there were components of important signaling pathways involved in embryogenesis and oogenesis such as Notch and TGF-β. Gene networks most significantly enriched with altered genes were identified, including a network related to Cellular Function and Maintenance, Molecular Transport, Organ Development, which is connected to the TGF-β signaling pathway and might be related to the effect of curcumin on pairing of adult worm pairs, egg production and viability of

worms. qPCR validation experiments with 7 genes have confirmed the expression changes detected with arrays. Here we suggest that transcriptional repression observed in Notch and TGF-β pathways could explain the effects on oviposition and egg development described in the literature.

Graphical abstract

Schistosoma mansoni has homologs of all 29 genes known to be affected in humans by Curcumin. Among them, 8 were up-/down-regulated in the parasite.



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Highlights

▸ 2374 differentially expressed genes of which 981 were up- and 1393 downregulated. ▸ Among them there were components of important signaling pathways. ▸ Pathways involved in embryogenesis and oogenesis such as Notch and TGF-β. ▸ Gene networks most significantly enriched with altered genes were identified. ▸ Repression in these pathways could explain effects on oviposition/egg development.

Previous Next

Keywords

Schistosoma mansoni; Curcumin; Microarray

1. Introduction

Schistosomiasis has been estimated to afflict as many as 207 million people in 76 countries,

with 779 million more being at risk of infection [1] causing more than 208,000 deaths per year [2]. Praziquantel is the drug of choice for the treatment of schistosomiasis. However, the existence of resistant strains [3], [4], [5], [6], [7], [8], the reduction in cure rates and the failure of treatment after praziquantel administration [9] reinforce the need to develop new safe and effective methods against schistosomiasis.

Research with medicinal plants constitutes a very viable strategy for drug discovery [10]. Curcumin is the active component from the rhizome of herb *Curcuma longa*, possessing many pharmacological and biological activities. This compound has anti-inflammatory, antioxidant, antiviral, anti-infectious, and anticarcinogenic effects [11], [12], [13], [14]. In addition, the use of curcumin as parasiticidal agents has been extensively studied. It has activity against *Leishmania* [15], [16], [17], [18], *Giardia lambia* [19] and *Trypanosoma* [20], [21]. The first studies about curcumin effects in *Schistosoma mansoni* showed the schistosomicidal effect of the oil extract of *C. longa* against *S. mansoni* infected mice [22], [23]. Our group described *in vitro* schistosomicidal activity of curcumin against *S. mansoni* adult worms [24]. Then, the schistosomicidal activity of curcumin *in vivo* was published [25]. Recently, El-Agamy et al. [26] showed that curcumin has potent antifibrotic activity in suppressing and reversing *S. mansoni*-induced liver fibrosis.

Considering these observations and knowing that this compound can modulate the expression of many genes, as described in other organisms, we proposed to investigate the effects of curcumin on *S. mansoni* adult worms through microarray analyses. These analyses could determine the possible signaling events related to parasite development, enabling the identification of possible targets for new therapeutical approaches against schistosomiasis.

2. Materials and methods

2.1. Ethics statement

In accordance with accepted national and international principles for laboratory animal welfare all experiments were authorized by the Ethical Committee for Animal Care of University of São Paulo.

2.2. Parasite culture and treatment

The LE strain of *S. mansoni* was maintained by passage through *Biomphalaria glabrata* snails and Balb/c mice. After 56 days, *S. mansoni* adult worms were recovered under aseptic conditions from mice previously infected with 200 cercariae by perfusion of the livers and mesenteric veins [27]. The worms were maintained in RPMI 1640 medium (Invitrogen) supplemented with penicillin (100 UI/mL), streptomycin (100 μg/mL) and 10% bovine fetal serum (Gibco). Adult worms were separated in two groups with 20 worm pairs each. The first,

control group was maintained in RPMI 1640 culture medium with 1% DMSO, since curcumin was dissolved in DMSO. In the second group, 15 μM curcumin (Sigma–Aldrich, St. Louis, MO, USA) was added to the culture medium. Both groups were maintained at 37 °C, 5% CO_2, during 24 hours. Under these conditions less than 20% of worm pairs had separated. Four biological replicas were assayed; for each replica, 5 groups of 20 worm pairs were pooled for each experimental condition (treated or untreated).

2.3. Total RNA extraction and microarray experiments

Total RNA from adult worms, treated or untreated with curcumin, was extracted with Trizol reagent (Invitrogen, Life Technologies Inc., Carlsbad, CA, USA), using the recommended protocol. After Trizol extraction, RNAs were treated with DNAseI (QIAGEN, Hilden, Germany) and subsequently purified using Qiagen RNAeasy mini kit (QIAGEN). The integrity of RNA samples was evaluated using microfluidic electrophoresis in the Bioanalyzer equipment (Agilent Technologies, Santa Clara, CA, USA) and quantified in the NanoDropTM 1000 spectrophotometer (Thermo scientific, Wilmington, DE, EUA). Gene expression analysis was performed using the 4 × 44 K oligoarray platform, an oligonucleotide microarray slide containing around 44,000 probes representing *S. mansoni* gene fragments, designed by Verjovski-Almeida et al. [28] and manufactured by Agilent Technologies; the platform probe annotation is available on gene expression omnibus (GEO) under the accession number GPL8606.

Parasites were kept in sets of 20 worm pairs each, and they were exposed to either curcumin or vehicle. Subsequently, five sets of worm pairs were pooled to comprise one biological replicate of either treatment or control experiments. We performed a total of four biological replicates, and for each biological replicate two technical replicates were obtained. For these two technical replicates we used 500 ng of total RNA each, to generate amplified cRNA that contained either Cy5- or Cy3-labeled dCTP according to the Agilent Quick Amp Labeling Kit (Agilent Technologies); this kit essentially produces a linear amplification and labeling of poly-A RNA with a T7-RNA polymerase. Hybridization was performed by the combination in one microarray of a technical replica from a treated sample labeled with one dye *vs.* a technical replica from a control sample labeled with the opposite dye (dye-swap approach); 825 ng of amplified Cy5- or Cy3-labeled cRNA was used for each hybridization. Overall, eight microarrays were hybridized (two slides of 4 × 44 k elements each). The slides were washed and processed according to Two-Color Microarray-Based Gene Expression Analysis (Quick Amp Labeling) Protocol (Agilent Technologies) and scanned on a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA, USA). Data were extracted using Feature Extraction software (Agilent Technologies). Raw data is available in GEO under the accession number GSE37647.

2.4. Processing and analysis of microarray data

Genes were considered as expressed when they had a mean signal intensity of the array spot that was significantly higher (2-sided *t*-test) than the mean signal of the local background (IsPosAndSig column from Feature Extraction data output). Genes were kept in further analyses when they were detected as expressed in at least 75% of all replicas from at least one biological condition (treated or control). The intensities were normalized by LOWESS algorithm [29]. Intensity correlations among different curcumin *vs* control microarrays ranged from 0.86 to 0.96 (average correlation = 0.92); among technical replicates of the same condition (treatment or control) the correlations were from 0.91 to 0.99 (average correlation = 0.97). Correlations among biological replicas were in the range from 0.91 to 0.99 (average correlation = 0.95).

The log 2 ratio of intensity data between treated and control for each gene was calculated. With these ratios, we used Significance Analysis of Microarray (SAM) [30] as the statistical test, to identify differentially expressed genes. We used SAM one-class approach and genes were considered as significantly differentially expressed at a cutoff *q*-value ≤ 0.03 and with a Fold Change > 1.5 (*i.e.*, log2 (Treated/control) > |0.58|). Hierarchical clustering of selected genes was generated using Spotfire Decision Site software (TIBCO Software Inc., Palo Alto, CA, USA). For a gene that was represented in the array by multiple probes, we picked a single representative probe (see the list of selected Feature Numbers in Supplementary Table S1) by selecting the probe with the lowest variation coefficient (obtained from intensity data of previous replicated experiments with the 44 k oligoarray). The ontology annotation of all differentially expressed genes (3433) was achieved using the Blast2GO online annotation pipeline [31] as described by Nawaratna et al. [32].

Functional analysis was performed using Ingenuity Pathway Analysis (IPA, http://www.ingenuity.com/). For this purpose we annotated *S. mansoni* genes encoding putative homologs to human proteins; the putative homolog should have similarity with a BlastX *e*-value lower than $10^{-1°}$ and coverage of at least 60% of the human homolog. The RefSeq number of each human homolog was associated to each *S. mansoni* gene and the expression data was uploaded to Ingenuity Pathway Analysis System version 7.6. We included all gene/protein relationships described as experimentally observed and/or predicted with high confidence.

2.5. Real-time quantitative PCR (q-PCR) validation of microarray data

One μg of DNAse-treated Total RNA was used as template to synthesize cDNA using the ThermoScript™RT-PCR System (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. cDNA synthesis was performed in the presence of oligodT primer and Reverse Transcriptase (Thermoscript RT-PCR® System, Invitrogen), according to the manufacturer's protocol; 0.5 μl of RT reaction was used for each PCR reaction. Reactions were carried out with SYBR green PCR Master Mix (Applied Biosystems) for 40 cycles in a total volume of 25 μl and

according to the manufacturer's instructions using the AB 7500 real-time PCR system (Applied Biosystems). The experiment was performed on 3 independent biological replicates; for each one, 3 technical replicas were performed. The results were analyzed by comparative CT method using the AB 7500 software. The transcript levels were normalized with α-tubulin endogenous control. Student *t*-test was used to calculate the statistical significance between samples. The observed expression differences were considered to be statistically significant at $p < 0.05$. Gene specific primers used are listed in Supplementary Table S2 and these were designed using Primer3 (http://primer3.sourceforge.net/) with default parameters.

The correlation of RT-qPCR expression data with microarray expression data was estimated as described in [33] for the seven genes that were selected for validation. For this purpose, we calculated for each of the seven genes the average log2 ratio (treated/control) among the six microarray data measurements (two technical replicas of each of three biological replicas) and it was transformed to fold change ($FC = 2^{\log2 \text{ ratio (treated/control)}}$). Next, we calculated for each of the seven genes the average fold change measured by qPCR among three biological replicas. Finally, the Pearson's correlation between the average Fold Change of gene expression measured by microarray and qPCR was calculated.

3. Results and discussion

It has been previously demonstrated that the *in vitro* incubation of *S. mansoni* with different doses of curcumin causes a reduction in viability of adult worms, a decrease in egg production and an increase in worm pairs separation [24]. Based on these observations, we aimed to identify the molecular targets that are involved in this phenotype by measuring gene expression changes induced by curcumin. Using an oligoarray composed of 44 k elements [28] we were able to detect the expression of 9910 genes in adult worms in culture. Applying SAM as a significance test (*q*-value < 0.03 and log ratio > |0.58|, *i.e.* a minimum 1.5-fold increase or decrease) we identified 2374 differentially expressed genes (Supplementary Fig. 1) of which 981 were up-regulated and 1393 were down-regulated upon curcumin treatment.

We annotated the genes that were represented in the array based on the *S. mansoni* genome project gene predictions [33] and on gene annotation in other species, available in GenBank. We found that among the set of differentially expressed genes, there were 1474 that were predicted in the genome (599 up-regulated by curcumin and 875 down-regulated); in addition there were 147 genes with homologs only in *S. japonicum* and in no other species (60 up-regulated and 87 down-regulated by curcumin); another 343 genes have a homologue in species other than *S. japonicum*, present in GenBank (152 are up-regulated and 191 down-regulated); 410 genes have no homologs in GenBank (No Match genes), and among them 170 are up-regulated and 240 have the opposite pattern of expression. Table 1 summarizes this result. Supplementary Table S3 contains the complete list of differentially expressed genes.

Table 1. General information about curcumin microarray experiments. Number of expressed genes detected in each category and differentially expressed genes affected by treatment of adult worms with curcumin.

***S. mansoni* gene category**	**Number of expressed genes**	**Number of differentially expressed genes***	**Number of upregulated genes***	**Number of downregulated genes***
Genes predicted in *S. mansoni* genome	6228	1474	599	875
Genes with *S. japonicum* predicted homologs	603	147	60	87
Genes with homologs in species other than *S. japonicum*	1401	343	152	191
No match genes (with no homologs in other species)	1678	410	170	240
TOTAL	9910	2374	981	1393

* Statistical cutoff parameters were q-value < 3% and minimal 1.5 fold change.

We highlight, among the differentially expressed genes, those that encode proteins related to signal transduction and proteins that are involved in important biological processes such as cell cycle regulation and possible host immune response evasion. Table 2 contains the list of highlighted down- and up-regulated genes affected by curcumin, respectively.

Table 2. Highlighted differentially down and up regulated genes in curcumin treated adult worms compared to the control group.

ProbeName	**Contig**	**Gene Smp**	**Gene annotation**	**Fold**	**Putative function**

				change*	
Q2_P17914	C800035.1	Smp_132920	Notch1 preproprotein [Homo sapiens]	–4.3	Signaling – Notch pathway
Q2_P05558	C810293.1	Smp_021920.2	paramyosin, putative	–4.0	Motor activity and DNA binding
Q2_P28534	C902343.1	Smp_010670	Abnormal long morphology protein 1 (Sp8), putative	–3.6	Calcium ion binding
Q2_P20876	C804080.1	Smp_049760	TGF-beta receptor, putative	–2.4	Signaling – TGF-beta pathway
Q2_P208776	C804080.1	Smp_049760	TGF-beta receptor	–2.3	Signaling – TGF-beta pathway
Q2_P29252	C903362.1	Smp_140800	Notch	–2.1	Signaling – Notch pathway
Q2_P32285	C908631.1	Smp_150080	transducin-like enhancer protein 1	–1.9	Signaling – Notch pathway
Q2_P12775	C909270.1	Smp_099440	bone morphogenetic protein antagonist noggin	–1.8	Signaling – Notch pathway
Q2_P21141	C804436.1	Smp_168390	tnf receptor-associated factor, putative	–1.6	Signaling – TNF- alpha pathway
Q2_P29493	C903695.1	Smp_124850	tyrosine kinase	–1.6	Signaling
Q2_P26504	C812054.1	Smp_050520	Notch	–1.6	Signaling – Notch pathway
Q2_P35186	C914212.1	Smp_132080	sugar transporter, putative	8.7	Sugar metabolism
Q2_P02262	C804648.1	Smp_170400	nicalin (M28 family)	6.3	Embryogenesis and oogenesis
Q2_P22056	C805734.1	Smp_113630	Antolefinin (Antolefinine), putative	6.3	Cell cycle regulation
Q2_P20751	C803921.1	Smp_133300	5′–3′ exoribonuclease, putative	5.5	Response to DNA damage stimulus
Q2_P33526	C910959.1	Smp_150140	PI3kinase, putative	4.5	mRNA processing, regulation of translation

Q2_P05911	C810678.1	Smp_012350.1	venom allergen-like (VAL) 11 protein	3.0	Possible host immune response evasion
Q2_P37765	C919184.1	Smp_079740	cullin-2 (cul-2), putative	1.5	ubiquitin-dependent protein catabolic process
Q2_P32366	C908755.1	Smp_082490	cyclin B, putative	1.5	Cell cycle

*

Genes with significant differential expression at q-value < 0.004 as analyzed by SAM (see Section 2) and with a fold-change higher than 1.5 or lower than –1.5. Negative fold-change refers to a fold-decrease in expression in curcumin-treated worms relative to control, whereas positive fold-change refers to fold-increase.

Among the differentially expressed genes there were components of important signaling pathways such as Notch and TGF-β, as discussed in detail below. Some of these genes and pathways (especially TGF-β) were already described as involved in *S. mansoni* development, embryogenesis and oogenesis processes in the parasite [34], [35].

Four of these genes are involved with Notch signaling pathway. The first two are Notch (Smp_140800, notch 2 [Homo sapiens] homolog, 28% coverage, 30% identity, 43% similarity and e-value = 10^{-78}), and Notch (Smp_050520, notch 3 human homolog, 44% coverage, 28% identity, 39% similarity and e-value = 10^{-15}), both down regulated by curcumin treatment. It is interesting that Transducin (Smp_150080, transducing like homolog, 95% coverage, 64% identity and 77% similarity, e-value = 10^{-154}), also known as Groucho, a Notch pathway co-repressor is down-regulated by curcumin treatment.

Notch signaling coordinates a wide range of fundamental processes and cellular programs including proliferation, apoptosis, migration, growth, and differentiation [36]. Notch receptors and their ligands have been first identified in *D. melanogaster* and *C. elegans* and studies showed their importance in oogenesis and embryogenesis of these organisms [37], [38], [39], [40].

The four mammalian Notch receptors are transmembrane proteins that are expressed on the cell surface [41]. The five mammalian Notch ligands are separated into two subgroups: Delta-like (Dll1, Dll3, and Dll4) or Serrate-like (Jagged1 and Jagged2), based on the structural similarity with their Drosophila homologues [42].

Since we found that in *S. mansoni* treated with curcumin two of the Notch receptor genes, as well as the Notch ligand gene and the co-repressor gene, were down-regulated, we suggest that the curcumin effects reported in the literature such as decreased oviposition and the arrested eggs development [24] may be caused by repression of the Notch pathway.

In *S. mansoni*, the pairing process, proliferation, differentiation of vitelline cells, expression of female-specific genes and embryogenesis are all regulated by the TGF-β pathway and protein tyrosine kinases [34]; TGF-β signaling also plays a major role in the sexual reproduction and embryogenesis of adult schistosome parasites; recently, a microarray analysis of TGF-β treated adult worms showed that biological functions such as morphology, development and cell cycle were affected by the human cytokine [43].

Our present results suggest that the TGF-β pathway signaling is decreased because two components of the pathway were down-regulated: TGF-beta receptor gene (Smp_049760) and Bone morphogenetic protein antagonist noggin (Smp_099440) message. Signaling by TGF-β members is through a family of transmembrane receptors that directly regulate the intracellular Smad pathway [34]. The second network most significantly enriched with genes altered by curcumin, as analyzed by the Ingenuity Pathway Analysis tool (see below), is related to Cellular Function and Maintenance, Molecular Transport, Organ Development and includes the ERC 1/2 complex that is a member of TGF-β signaling pathway, an important pathway for development as discussed before. MAPKK3 is a member of the ERC 1/2 complex, is indirectly down-regulated by curcumin in human cells and was observed here as down-regulated in schistosomes. In the same network we observed another gene that is known to be indirectly regulated in humans by curcumin, KCNB1 (potassium voltage-gated channel) and it was down-regulated in *S. mansoni*, an opposite effect to that described in human cells. Taken together, the observed changes in the expression level of TGF-β signaling elements are entirely consistent with the hypothesis that this pathway is a major player in the physiological effects previously described for curcumin, namely reduced pairing of adult worm pairs, reduced egg production and viability of worms. In this context, TGF-β pathway represents a potential prime target for the development of new strategies for the treatment and prevention of schistosomiasis [44].

We evaluated the GO categories representation among the differentially down-regulated genes (Supplementary Fig. S2) and up-regulated genes (Supplementary Fig. S3); a pie-chart representation is shown for each ontology category, namely “Biological Process”, “Molecular Function” or “Cellular Component”. No GO categories were identified as significantly enriched with differentially expressed genes.

We performed a functional analysis using Ingenuity Pathway Analysis (IPA) software. IPA provides the biological context for gene expression changes by integrating available literature information on model organisms (human, mouse, rat) regarding molecular and chemical interactions, cellular phenotypes as well as about signaling and metabolic pathways. IPA provides pre-computed libraries of canonical pathways, which are well-characterized metabolic and cell signaling pathways that are based on the curated literature, have a directionality of flow, have been generated prior to our data input, and do not change upon our data input; in this case IPA computes the canonical pathways enriched with up- or down-

regulated genes. In addition, IPA computes what are called enriched networks, which are generated *de novo* based on our input data, do not have directionality, and typically contain molecules involved in several pathways.

With IPA functional analysis we were able to identify interaction networks that were statistically enriched with genes that showed altered expression caused by curcumin, and here we highlight the five most significant (p-values from 10^{-52} to 10^{-35}). The first network (p-value = 10^{-52}) is associated to functions such as DNA replication, recombination and repair, energy production and nucleic acid metabolism; the second network (p-value = 10^{-50}) is associated with Cellular Function and Maintenance, Molecular Transport, Organ Development; the third network (p-value = 10^{-48}) is related to Gene Expression, Protein Synthesis, RNA Damage and Repair; the fourth network (p-value = 10^{-41}) is related to functions such as Tissue Morphology, Cell-To-Cell Signaling and Interaction, and the fifth network (p-value = 10^{-35}) is related to Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance. A list of differentially expressed genes that comprise these five networks is in Supplementary Table S4. In Supplementary Fig. S4, we highlight the second network, which is related to functions such as Cellular Function and Maintenance, Molecular Transport, Organ Development, which might be related to the effect of curcumin on pairing of adult worm pairs, egg production and viability of worms [24]

IPA pointed to twenty-nine genes already described in the literature as directly or indirectly affected by curcumin in model organisms and whose *S. mansoni* homologs were identified in *S. mansoni*; among them, eight were differentially expressed by exposure of the parasite to curcumin. Fig. 1 shows these genes and their relations with curcumin. Supplementary Table S5 lists which type of curcumin relation is described for each of these genes in human cells; notably, in humans curcumin either causes a change in their gene expression levels (seventeen of them) or in the function of the protein encoded by these genes (twelve of them) (see Fig. 1). For two out of the twenty-nine we do not have expression data because they are not represented in the array (HDAC2 and CASP7); it is worth to note that for six out of seventeen homolog genes that were described in humans to have expression changes indirectly mediated by curcumin, we detected a change in expression in *S. mansoni*. Among them, three genes, namely MAPK3, POLR2C and SOD1 had expression changes concordant with the changes described in the literature for human cells (please see Supplementary Table S5). Among the twelve genes with described effect of curcumin on the activity of the encoded protein in humans, two of them showed changes in the expression level of the genes in *S. mansoni*; they are SRC (for which the drug is described to directly interact with the encoded protein) and PARP1.

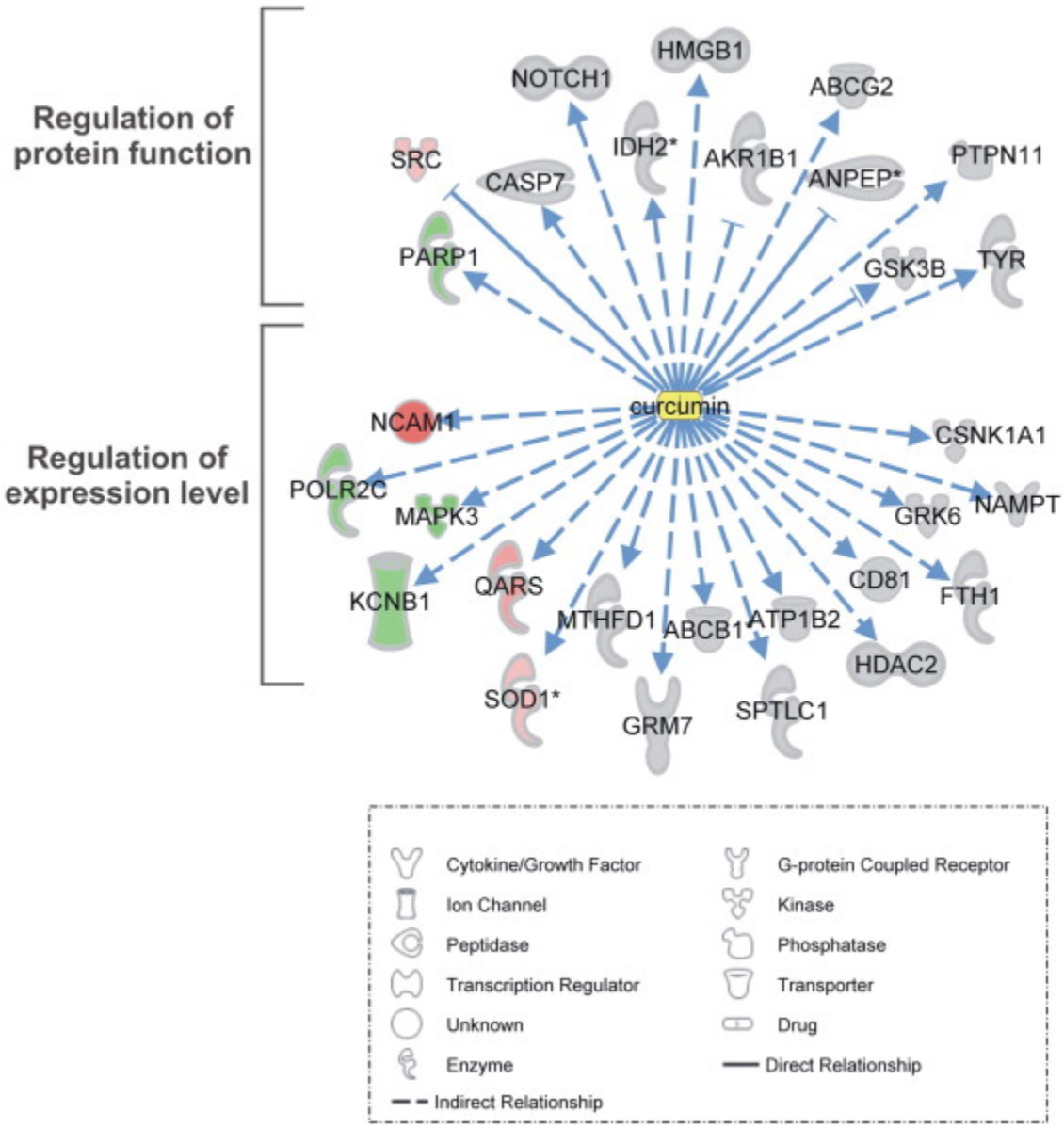

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Fig. 1. Direct and indirect gene interaction of curcumin with *S. mansoni* homologs. The figure shows *S. mansoni* homolog genes with curcumin-related effects already described in human cells. In the upper part are the genes for which an effect of curcumin on the activity of the protein encoded by the gene has been described in humans; on the lower part are the genes for which an effect of curcumin on the level of expression has been described in humans. The effect of curcumin on the level of the expressed gene in *S. mansoni*, as detected in the present work, is shown by color: genes that were induced by treatment are shown in red and those repressed are in green.

It is interesting to note the effect of curcumin on SRC (v-src sarcoma (Schmidt-Ruppin A-2)

viral oncogene homolog); in human cells curcumin directly interacts with SRC and increases its inhibition [45]; here we observed an up-regulation in the expression of this gene, possibly as a parasite's response to compensate for drug inhibition of the activity of the protein. Recently it was documented that SRC mediates the activation of Notch 1 (processing of full length Notch 1 by the interaction with Furin molecule) in pancreatic cancer cells, and the interaction Notch1-Furin that is mediated by SRC is induced by growth factors such as TGF-β [46]. Here one more time we point to the relevance of TGF-β and Notch signaling pathways in the biological effects that the drug has on the parasite.

We selected seven genes to validate the expression changes measured in the microarray experiments using RT-qPCR approach. These genes were chosen based on their potential role in the parasite's biology and because their change in expression possibly correlates with the effects previously described of curcumin treatment. Reverse-transcription real-time PCR analysis confirmed the expression changes induced by curcumin in all selected genes (Fig. 2). We calculated Pearson's correlation (see Section 2) between the microarray and Real time PCR average expression data (average fold change) and a positive correlation of 0.99 was obtained.

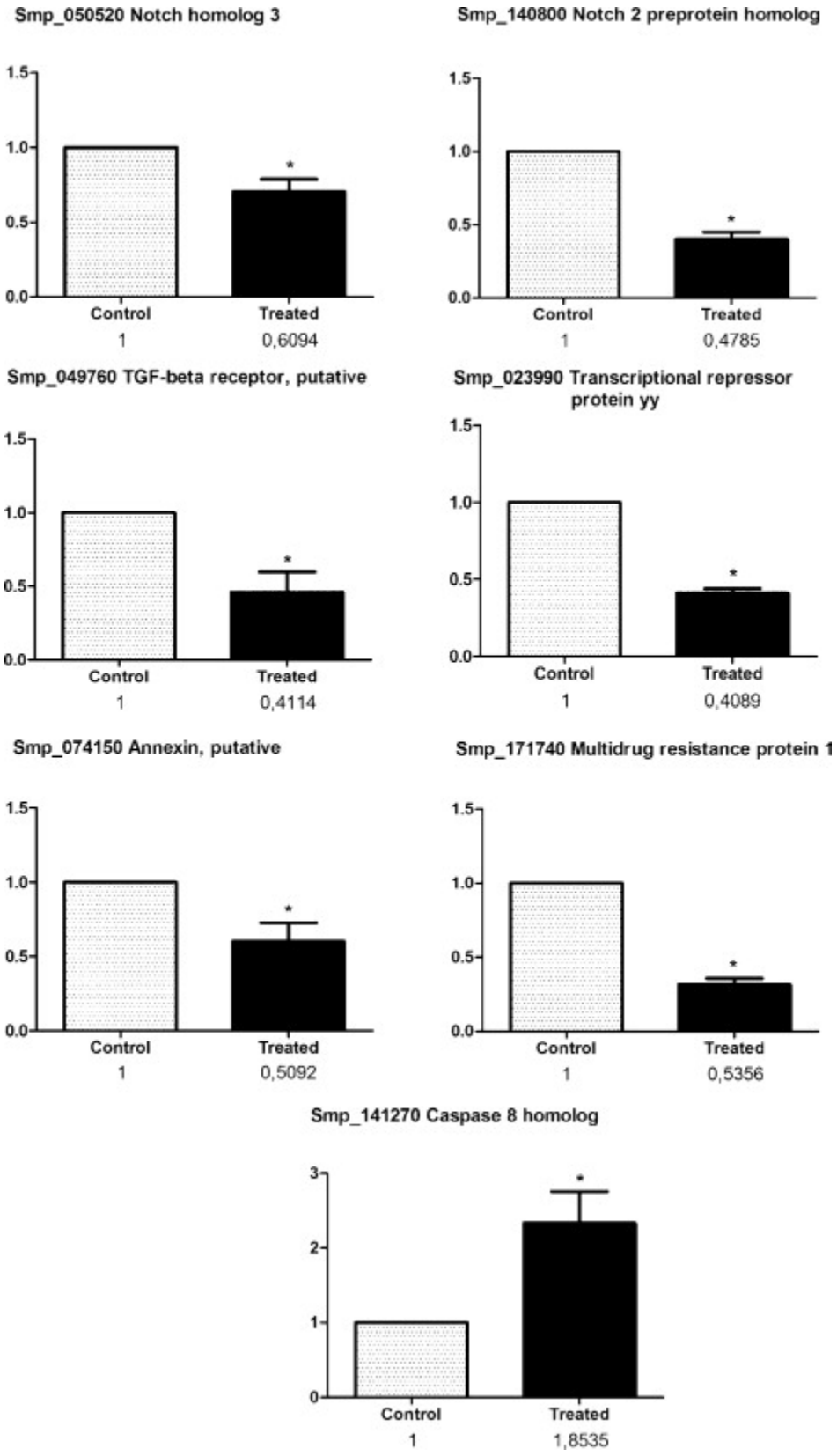

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Fig. 2. Real-time PCR validation of microarray. Validation is shown using a subset of differentially expressed genes in curcumin treated *S. mansoni* adult worms compared to the

control group. Reverse-transcription-qPCR data, expressed as fold change (normalized to control group), are presented as bar graphs, while the corresponding microarray data (fold change) are shown below in numbers.

The top fifty genes with the highest expression changes (twenty-five top up regulated and twenty-five top down regulated) are listed in Supplementary Table S6. It is noteworthy that among these fifty genes with highest expression changes, nine are found only in *S. mansoni* ("no hits" in other organisms), and another thirteen are annotated either as "Hypothetical protein" or as "expressed genes"; *i.e.* twenty-two out of fifty top differentially expressed genes (44%) have annotation that does not provide any clue about their function and might be good candidates to be preferentially explored in the future.

4. Conclusions

Further studies are needed to understand how the TGF-β and Notch signaling pathways identified in the present work interact with each other and how they mediate the biological processes affected by curcumin; these could be direct or indirect effects of the drug. Identification of key points of regulation will open new directions in the development of therapeutic targets. Curcumin is a possible drug for treatment of schistosomiasis and here we took the first steps toward elucidating the molecular mechanism involved in its activity.

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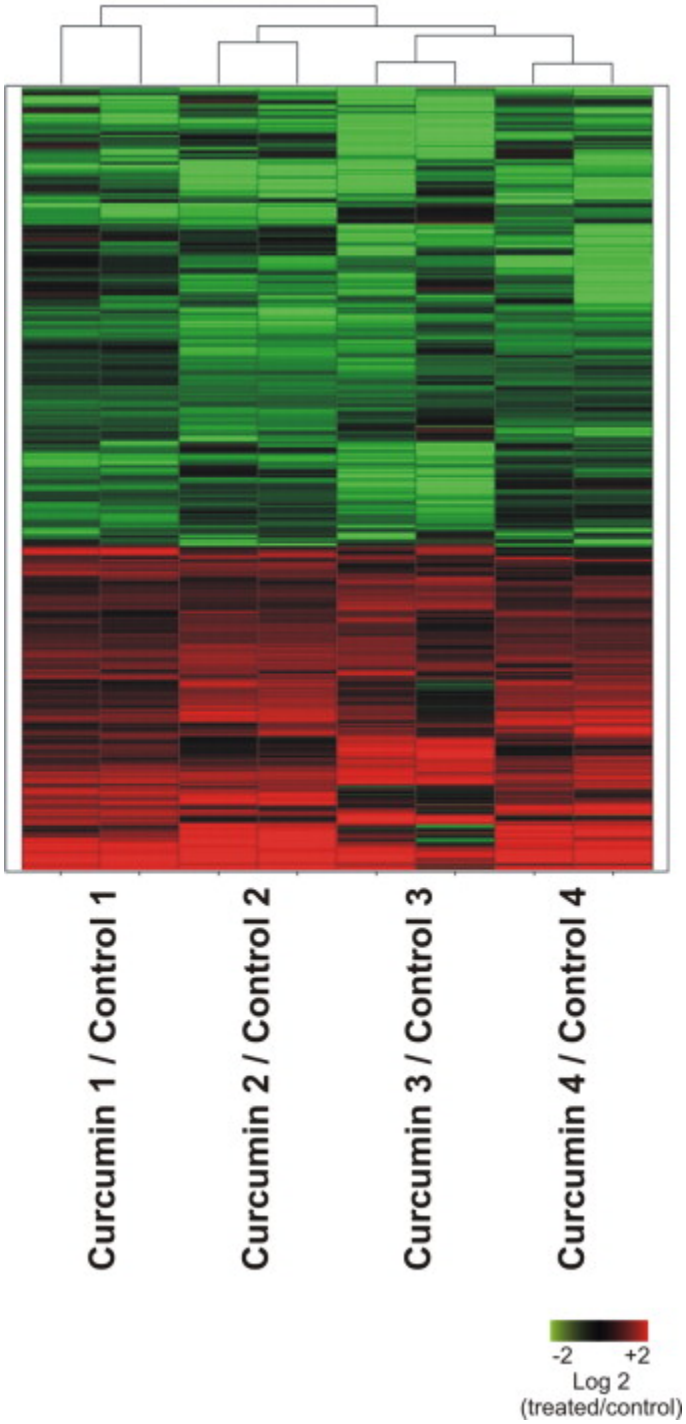
Appendix A. Supplementary data

The following are Supplementary data to this article:



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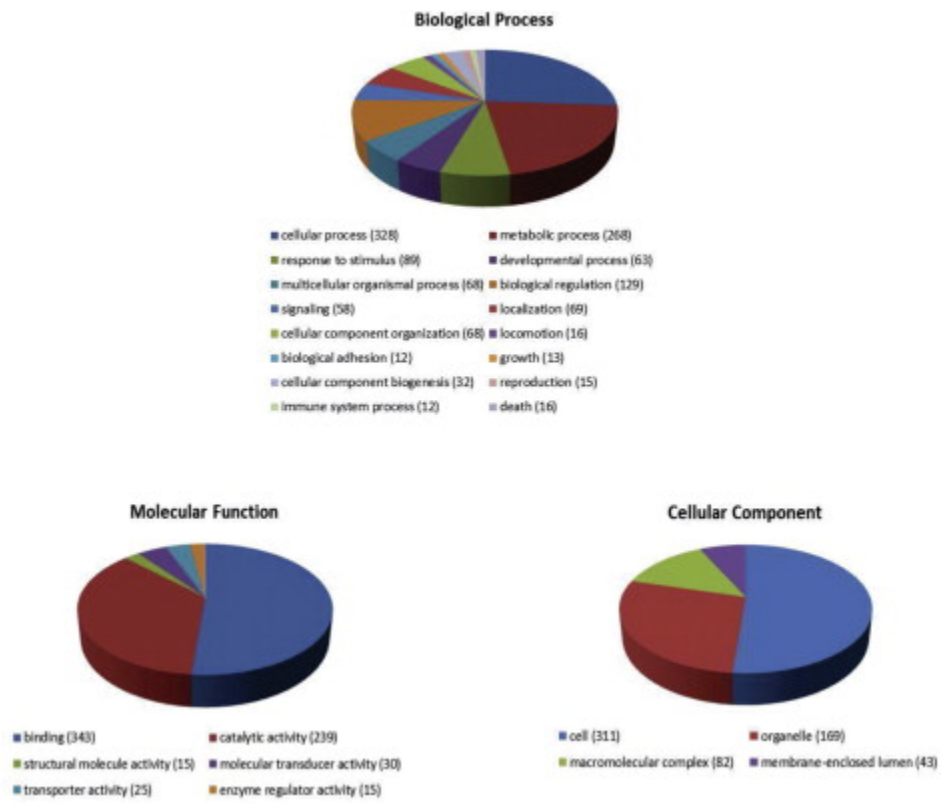

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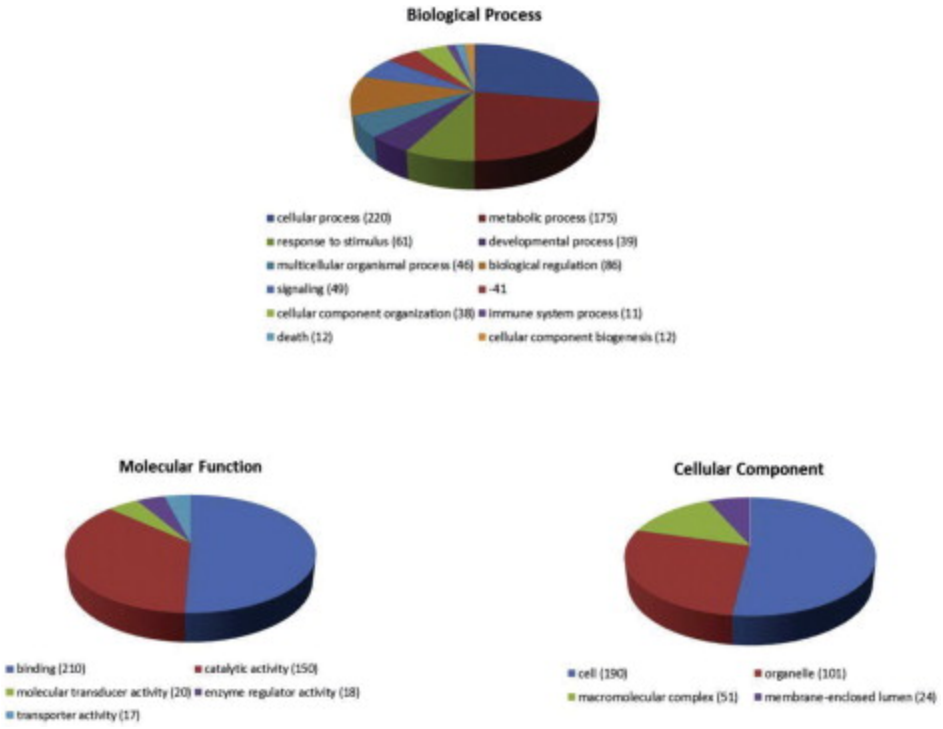

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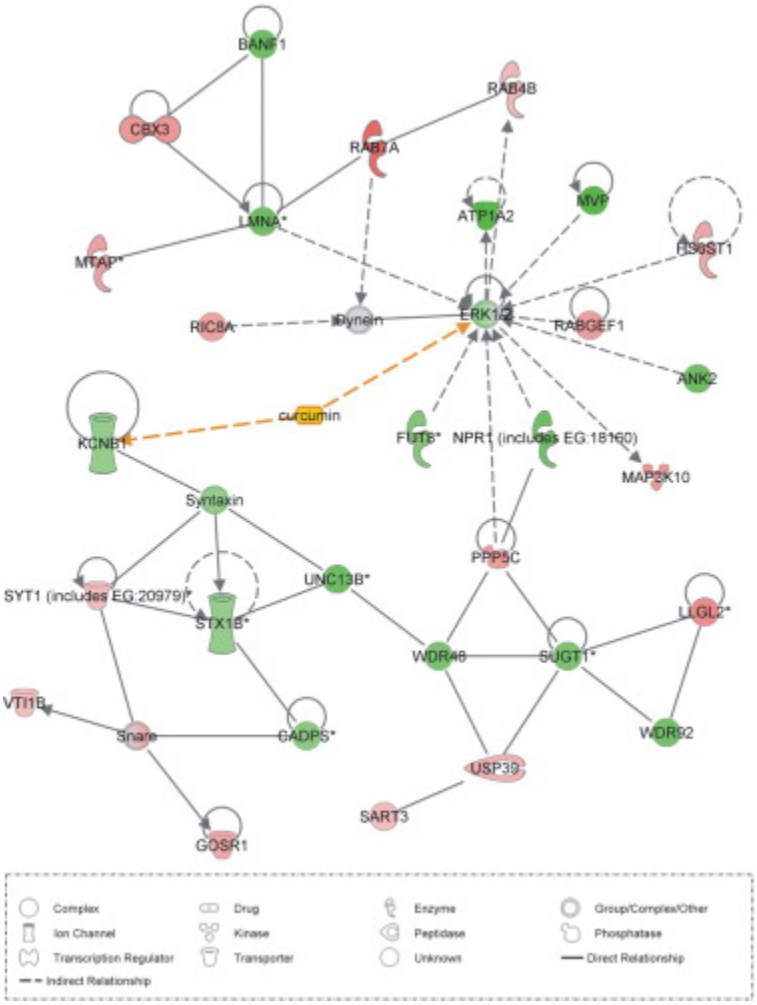

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