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Identification of the differentially expressed genes in SP2/0 myeloma cells from Balb/c mice infected with *Trichinella spiralis*

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ABSTRACT

To study the molecular mechanism of suppressed growth caused by *Trichinella spiralis*, an SP2/0 myeloma model was established using parasite-infected Balb/c mice. Suppression subtractive hybridization (SSH) was then utilized to identify differentially expressed genes between tumor cells from the infected and non-infected mice. On the 11th day after infection, 2×10^6 SP2/0 myeloma cells were subcutaneously inoculated into 6–8 week old female Balb/c mice in both the experimental and control groups. Twenty-eight days after tumor cell inoculation, the mice were euthanized and the sizes and weights of the tumors were measured. Messenger RNA was isolated and used to perform SSH. Putative differentially expressed genes were identified, sequenced and analyzed by BLASTn. Among the sequences detected which ranged in size between 180 and 850 bp, genes encoding RpL41, NKTR, Rbbp4 and ANXA2 were enriched and considered possible proteins involved in tumor growth inhibition.

1. Introduction

Although *Trichinella spiralis* is a parasitic pathogen that causes harm to human and animal health, previous work suggests that *T. spiralis* can also be beneficial. Tumorigenesis is a complicated process involving the expression of multiple genes and the formation of complex interactions. Substantial research has been performed using high throughput techniques to screen for candidate genes responsible for different aspects of tumorigenesis such as metastasis. In addition, previous investigations have shown that *T. spiralis* can enhance the host resistance to tumors by inhibiting the proliferation of tumor cells in vitro, and the growth of tumor tissues transplanted into rats. However, the molecular mechanisms underlying this anti-tumor effect remain poorly understood.

PCR-based suppression subtractive hybridization (SSH) techniques are highly sensitive for identifying differentially expressed genes (Diatchenko et al., 1996, 1999; Kuang et al., 1998; Harms et al., 2002; Rebrikov et al., 2004). In the present study, an SSH cDNA library was successfully generated using SP2/0 myeloma cells retrieved from uninfected and *T. spirali*-infected Balb/c mice. Preliminary data presented herein show substantial variation in tumor gene expression in the presence of *T. spiralis*. A more comprehensive study of the genes enriched in this SSH library can help better understand the molecular mechanisms involved in the anti-tumor effects induced by *T. spiralis*.

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2. Materials and methods

2.1. Mice

Six to eight week old female Balb/c mice were purchased from the Center of Experimental Animals, Jilin University. Procedures for handling mice were performed under the regulations of the university. The infection of mice with 450 L1 and the recovery of *T. spiralis* were performed according to a previously reported procedure (Wakel and Wilson, 1977).

2.2. SP2/0 cell culture, inoculation and sample preparation

SP2/0 murine myeloma cells (ATCC line) were cultured in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum and 100 U of penicillin–streptomycin/ml under a humidified atmosphere with 5% CO_2 at 37 °C. Forty inbred Balb/c mice were randomly divided into two equal groups one of which received 450 L1. At 11 days post infection (dpi), 2×10^6 SP2/0 myeloma cells were subcutaneously inoculated into all 40 mice. The mice in both groups were then euthanized on the 20th day after inoculation with the cells. The implanted tumors were removed under sterile conditions at the time of necropsy. The samples were cut into 100 mg portions and stored at −80 °C.

2.3. Suppression subtractive hybridization (SSH)

The suppression subtraction hybridization procedure was carried out according to the manufacturer's instructions in the PCR-Select™-cDNA Subtraction Kit (Clontech, USA) using 2 μg each of mRNA from the tester (*T. spiralis*-infected group) and the driver (uninfected control group) to synthesize cDNA. Tester and driver cDNAs were digested with RsaI. The tester cDNA was split into two groups, and each was ligated with a different adapter. In the first hybridization reaction, an excess of driver was added to each sample of tester. The samples were heat denatured and allowed to anneal. Because of the second-order kinetics of hybridization, the concentration of high and low abundance sequences is equalized among the single-stranded tester molecules. At the same time, single-stranded tester molecules were significantly enriched for differentially expressed sequences. During a second hybridization, the two primary hybridization samples were mixed together without denaturation. Presumably, only the remaining equalized and subtracted single-stranded tester cDNAs can re-anneal to form double-stranded tester molecules with different ends. This population of molecules was treated with DNA polymerase to fill-in the ends then subjected to nested PCR with two adapter-specific primer pairs. The resulting PCR products were amplified with primers encoding G3PDH (a housekeeping gene) at 18, 23, 28, 33 cycles to validate subtraction efficiency. Products of the secondary PCRs were cloned into a pT Adv vector (Clontech) and the resultant ligation products were transformed into competent DH5α *Escherichia coli* cells.

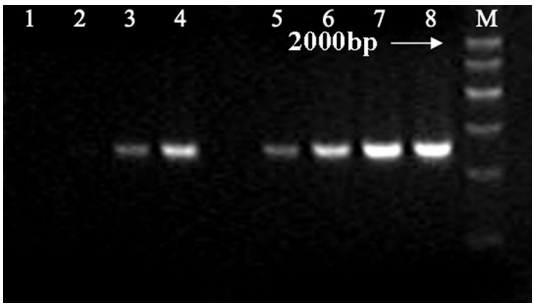

Fig. 1. Efficiency of the SSH procedure based on GAPDH amplification. (M) DNA marker DL2000; (1)–(4) PCR products from the subtracted cDNA; (5)–(8) PCR products from the non-subtracted cDNA. Lanes 1 and 5 = 18 cycles; lanes 2 and 6 = 23 cycles; lanes 3 and 7 = 28 cycles; lanes 4 and 8 = 33 cycles.

2.4. Identification of the subtracted clones

Plasmids of candidate positive clones from the SSH library were isolated and amplified by PCR using nested primers 1 (5′-TCGAGCGGCCGCCCGGGCAGGT-3′) and 2 (5′-AGCGTGGTCGCGGCCGAGGT-3′) according to the manufacturer's instructions (Clontech). The PCR products were detected by agarose gel electrophoresis. Twenty randomly selected clones were sequenced and used to search (BLASTn) the National databases (National Institutes of Health, Bethesda, MD, NCBI).

3. Results and discussion

3.1. Effects of tumor growth suppression

Physiological changes to tumor growth as a result of the presence of *T. spiralis* were evaluated by determining the size and weight of tumors in each group. The mean volume and weight of the control group ($N = 10$) were $5.364 \pm 0.543\ cm^3$ and 1.552 ± 0.327 g, respectively. The corresponding mean volume and weight of the infected group ($N = 10$) were $1.6642 \pm 0.102\ cm^3$ and 0.750 ± 0.142 g, respectively. The data show that the sizes and weights of the tumors from the infected group were significantly lower ($P < 0.01$) that those taken from control mice where the tumor control rate was estimated at 46%.

3.2. Differential screening and analysis of the subtraction library

In this study, the tester cDNA was from myeloma cells in the presence of *T. spiralis* and the driver cDNA was from myeloma cells in the absence of *T. spiralis*, Subtraction efficiency showed that the PCR products of G3PDH in the non-subtracted library were visible after only 18 cycles whereas 28 cycles were required in the subtracted library (Fig. 1). The abundance of non-differentially expressed genes was effectively reduced, which indicated that the construction of the subtraction library was successful.

Using the SSH technique, we initially obtained 30 clones with inserts ranging from 150 bp to 850 bp from a total of 75 putative differentially expressed clones by nested