Available online at www.sciencedirect.com
ScienceDirect
Experimental Parasitology 118 (2008) 134–138
Experimental Parasitology
www.elsevier.com/locate/yexpr

Research brief

Toxoplasma gondii: Effect of infection on expression of 14-3-3 proteins in human epithelial cells

Fernando P. Monroy *

Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, USA

Received 10 May 2007; received in revised form 26 June 2007; accepted 16 July 2007
Available online 3 August 2007

Abstract

14-3-3 Proteins are expressed in most eukaryotes organisms and play varied and crucial roles in a wide range of regulatory processes. In mammalian cells, seven 14-3-3 isoforms have been identified. However, it is not known what effect infection has on 14-3-3 isoform expression. In this study human colonic carcinoma cell lines were infected with *Toxoplasma gondii* for 24 h and expression of 14-3-3 proteins was determined by RT-PCR. HT-29 cells only expressed 3 out of the 7 isoforms while 5 and all 7 isoforms were found in HCT-116 and Caco-2 cells, respectively. Infection had little or no effect in the expression of 14-3-3γ, ε, σ, and ξ; but in HCT-116 cells induced expression of 14-3-3η and σ, while 14-3-3β, η, and ξ were induced in HT-29 cells. If 14-3-3 proteins are involved in cell survival and/or prevention of parasite replication, longer incubation times may be required as no differences in percentage of infection were found among the cell lines at 24 h post-infection.
Published by Elsevier Inc.

Index Descriptors and Abbreviations: 14-3-3 Isoforms; RT-PCR; *Toxoplasma*; Colonic epithelial cells; Colon cancer cells

1. Introduction

14-3-3 Proteins are a family of acidic dimeric proteins expressed in all eukaryotic organisms, including parasites (van Hemert et al., 2001; Siles-Lucas and Gottstein, 2003). 14-3-3 Proteins are highly conserved in amino acid sequences from yeast to mammals. Seven isoforms encoded by seven distinct genes are identified in mammals while more than 10 isoforms are identified in plants (Wu et al., 1997), and two isoforms are identified in yeast (Ford et al., 1994; van Heusden et al., 1995). In mammals the function of 14-3-3 proteins is not completely understood but they play varied and crucial roles, including signal transduction, cell cycle control, stress responses, vesicular transport, DNA replication, cytoskeleton organization, prevention of apoptosis, and malignant transformation (Baldin, 2000; Fu et al., 2000; van Hemert et al., 2001; Hermeking et al., 1997; Muslin and Xing, 2000; Tzivion and Avruch, 2002; Tzivion et al., 2006).

Homologs of 14-3-3 proteins have been reported in helminth and protozoan parasites (Siles-Lucas and Gottstein, 2003). However, the number of 14-3-3 isoforms, their function, cellular localization, and expression pattern has been studied in only a few of them. Their crucial role in biological processes such as parasite proliferation and survival has made them attractive targets for vaccination (Zhang et al., 2000; Schechtman et al., 2001; Zhang et al., 2001; Siles-Lucas and Gottstein, 2003). In *Toxoplasma gondii* 14-3-3 isoforms have been reported in feline enteroepithelial gametocyte stages (Koyama et al., 2001) and in tachyzoites (Assossou et al., 2003). Tachyzoites contain two isoforms, one is mainly cytosolic with a small fraction membrane-associated, while the other is only present in the lipid rafts of detergent-resistant membranes (Assossou et al., 2003).

Expression of 14-3-3 isoforms is generally increased in human cancer cells (Qi and Martinez, 2003; Tzivion et al., 2006) but their regulation by infection by an intracellular parasite is not known. In this study, human colonic

* Corresponding author. Fax: +1 928 523 7500.
E-mail address: Fernando.Monroy@nau.edu

0014-4894/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.exppara.2007.07.008

carcinomas epithelial cells were infected with the obligated protozoan parasite *Toxoplasma gondii* to determine 14-3-3 isoform regulation by infection.

2. Materials and methods

Three human colorectal carcinomas, HT-29 (ATCC, HTB-38), Caco-2 (ATCC, HTB-37), and HCT-116 (ATCC, CCL-247) were used in these studies. These cells are well established models for studies of intestinal epithelial cell biology. Cells were maintained in DMEM supplemented with 10% FBS, 2 mM sodium pyruvate, 0.1 mM MEM non-essential amino acids solution, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine at 37 °C in a humidified atmosphere of 5% CO_2. Tachyzoites of the virulent *T. gondii* RH strain were maintained in vitro by infection of human foreskin fibroblasts. Parasites were collected and purified using a 3-μm polycarbonate membrane filter (Millipore, Bedford, MA, USA) (Monroy et al., 1999; Aviles and Monroy, 2001). Cells (1×10^5/ml) were grown at 70% confluence in 6 well plates until exposed to *T. gondii*. Infection of colonic epithelial cells was performed at a 1:2 cells to parasites ratio and incubated for up to 24 h. Cells were collected at 3, 6, 9, 12, and 24 h after infection. A set of wells was used to determine percentage of infection after 24 h. In these wells, cells were grown on 1 cm round tissue culture cover slips. Cover slips were fixed in methanol, stained with DiffQuick (Baxter, Miami, FL, USA), and observed under 100× magnification. At collection times, cells were washed briefly with sterile PBS to remove non-intracellular parasites before processing for RNA collection. Total RNA was isolated from cultured cells using Trizol (Gibco, Rockville, MD, USA) according to the manufacturer's instructions. Reverse transcription was carried out at 42 °C for 50 min using 5 μg of each RNA with 0.5 μg/μl Oligo(dT)12–18, 10 mM dNTP mix, 5× RT buffer, 50 mM $MgCl_2$, 0.1 mM DTT, RNase inhibitor and 50 U of M-MuLV reverse (Fermentas, Hanover, MD, USA). Primers that would specifically amplify each of the 7 human 14-3-3 isoforms according to their cDNAs from GenBank are shown in Table 1. The PCR consisted of 12.5 μl 2× PCR mix (Fermentas, Hanover, MD, USA), 1.5 μl RT product, 1 μl each of forward and reverse primers (10 mM), and water to 25 μl. The reaction was carried out for 30 cycles of 94 °C for 40 s, 55 °C for 50 s and 72 °C for 60 s. Five microlit expression was performed in a FluorChem FC2 (Alpha Innotech Corporation, San Leandro, CA, USA). At each time point pixels for the isoforms were compared to those of β-actin and expressed at percent relative expression.

3. Results

Total RNA was isolated and RT-PCR was carried out using isoform specific primers. These primers have been used for amplification of isoforms in breast tissue samples and their expected amplification fragment sizes have been corroborated by sequencing (Martinez personal communication). There is a different pattern of 14-3-3 isoforms in normal and cancerous tissues (Qi and Martinez, 2003). All isoforms were detected in Caco-2 (Fig. 1c) while 14-3-3γ, ε, σ, and θ were minimally expressed by HT-29 cells (Fig. 1b). HTC-116 cells did not express 14-3-3η, and θ (Fig. 1a). Infection with *T. gondii* in Caco-2 cells primarily decreased the expression of 14-3-3η, θ and σ with this effects taking place after 12 h for 14-3-3θ and 24 h for 14-3-3σ (Fig. 1c). In HT-29 cells infection resulted in induced expression of 14-3-3ξ at 6 h while 14-3-3γ and η were expressed at 12 h after infection (Fig. 1b). In HTC-116 cell infection induced expression of 14-3-3θ only at 6 h and 14-3-3η was minimally detected at 3 h after infection (Fig. 1a). This expression was evident when densitometry analysis was performed to obtain relative expression of all isoforms (Fig. 2). Infection had no effect in the expression of 14-3-3ε in all cell lines which was constitutively expressed in control as well as in infected cells throughout the study. When cells were investigated to determine percentage of infection, we found no significant differences ($n = 3$; $p < 0.19$) in the number of infected cells among the three cell lines at 24 h post-infection; although, HCT-116 and Caco-2 cells had lower number of infected cells (Fig. 3).

4. Discussion

We have investigated the effect on *T. gondii* infection on 14-3-3 protein expression in human colonic epithelial cells. All three cell lines used in this study have been well charac-

Table 1
List of primers used for amplification of all seven 14-3-3 isoforms

Accession number	14-3-3 Isoform	Forward primers	Reverse primers	Expected size (bp)
X57346	β	5′-CATTCGGCTGTGGATAGAGA-3′	5′-CATCTGCTGCTTCTTCTCATTCC-3′	269
AM024334	γ	5′-CAGTGTCCTCCTCCTTCTCC-3′	5′-CTGGCACACAGCCTCCAACTCCTTCT-3′	291
NM_0067613	ε	5′-GAGCGATACGACGAAATGGTG-3′	5′-CCTTGGACTCGCCAGTGTTAG-3′	263
X80536	η	5′-CTAGCGAGCCAGCGGTGTGA-3′	5′-CTGTCTCCAGCTCCTTCTCAATCTTCTCCC-3′	287
AF029082	σ	5′-AGAGACACAGAGTCCGGCATTGG-3′	5′-TCCACCTTCTCCCGGTACTCACGC-3′	290
NM_006826	θ	5′-CGTGAAGCTCTCGAGGCTCCT-3′	5′-AATTCCAGCACCGTGGTGCAGATG-3′	344
U28964	ξ	5′-GCCTGTGAGCAGCGAGATCC-3′	5′-AGCATGGATGACAAATGGTC-3′	842
AK223055		5′-GTGGGGCGCCCCAGGCACCA-3′	5′-CTCCTTAATGTCACGCACGATTTC-3′	516

Sequences obtained from Qi and Martinez (2003).