Available online at www.sciencedirect.com
ScienceDirect
Virology 364 (2007) 10–20
VIROLOGY
www.elsevier.com/locate/yviro

Rapid Communication

Transcriptional profile of Rous Sarcoma Virus transformed chicken embryo fibroblasts reveals new signaling targets of viral-*src*

Kathryn Masker [a], Alicia Golden [a], Christian J. Gaffney [a], Virginia Mazack [a], William F. Schwindinger [a], Weizhou Zhang [b], Lu-Hai Wang [b], David J. Carey [a], Marius Sudol [a,c,*]

[a] *Weis Center for Research, Danville, PA, USA*
[b] *Department of Microbiology, Mount Sinai School of Medicine, New York, NY, USA*
[c] *Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA*

Received 2 February 2007; returned to author for revision 5 March 2007; accepted 15 March 2007
Available online 19 April 2007

Abstract

Transformation of chicken fibroblasts in vitro by Rous Sarcoma Virus represents a model of cancer in which a single oncogene, viral *src*, uniformly and rapidly transforms primary cells in culture. We experimentally surveyed the transcriptional program affected by Rous Sarcoma Virus (RSV) in primary culture of chicken embryo fibroblasts. As a control, we used cells infected with non-transforming RSV mutant td106, in which the *src* gene was deleted. Using Affymetrix GeneChip® Chicken Genome Arrays, we report 811 genes that were modulated more than 2.5 fold in the virus transformed cells. Among these, 409 genes were induced and 402 genes were repressed by viral *src*. From the repertoire of modulated genes, we selected 20 genes that were robustly changed. We then validated and quantified the transcriptional changes of most of the 20 selected genes by real-time PCR. The set of strongly induced genes contains vasoactive intestinal polypeptide, MAP kinase phosphatase 2 and follistatin, among others. The set of strongly repressed genes contains TGF beta 3, TGF beta-induced gene, and deiodinase. The function of several robustly modulated genes sheds new light on the molecular mechanism of oncogenic transformation.

Keywords: Avian sarcoma; Schmidt-Ruppin strain of RSV; *src* oncogene; Protein-tyrosine kinase; Vasoactive intestinal polypeptide; MAP kinase phosphatase 2; Follistatin

Introduction

Transformation of primary chicken fibroblasts (CEFs) in vitro by Rous Sarcoma Virus (RSV) continues to represent an important experimental model of cancer (Martin, 2004). The transforming gene of RSV, the viral *src* oncogene (v-*src*), is an activated and overexpressed protein-tyrosine kinase responsible for a number of molecular events that govern rapid and dramatic phenotypic changes observed in transformed host cells (Jove and Hanafusa, 1987; Blume-Jensen and Hunter, 2001; Darnell, 2002; Frame, 2002). Transformation-specific changes in cell morphology, proliferation and anchorage independence of growth are accompanied by changes in gene expression. In the past, a number of laboratories reported identification of individual genes that were either induced or repressed by the v-*src* oncogene at the level of transcription (Howard et al., 1978; Hendricks and Weintraub, 1981; Fagan et al., 1981; Sugano et al., 1987; Bedard et al., 1987; Jahner and Hunter, 1991; Dehbi and Bedard, 1992; Herault et al., 1992; Frankfort and Gelman, 1995). With the recent completion of the chicken genome sequence (Wallis et al., 2004; Hillier et al., 2004) and the subsequent availability of Affymetrix GeneChip® Chicken Genome Arrays that represent the full complement of the genome, we experimentally surveyed the transcriptional program affected by RSV transformation in the syngeneic system of Schmidt-Ruppin strain of RSV (RSV-SR) and primary culture of CEFs. As a control, we used CEFs infected with non-transforming RSV (td106) derived from the Schmidt-Ruppin strain of RSV in which the *src* gene was deleted (Wang

* Corresponding author. Laboratory of Signal Transduction and Proteomic Profiling, Weis Center for Research, 100 North Academy Avenue, Lab 202, Danville, PA 17822-2608, USA.
E-mail address: msudol1@geisinger.edu (M. Sudol).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.03.026

et al., 1984). Thus, the isogenic pair of viruses differed only in the *src* gene, but not in the replicative genes, allowing one to score for transcripts relevant for *src* transformation. The rationale for the global analysis of transcriptional changes caused by v-*src* was to provide better understanding of the mechanism of transformation, taking advantage of a cancer model in which a *single* oncogene uniformly and rapidly transforms *primary* cells in culture (Brugge and Erikson, 1977).

Using GeneChip® Chicken Genome Arrays that cover 32,773 transcripts corresponding to 28,418 predicted genes from the Ensembl annotation of the complete genome, we report 409 genes that are induced and 402 genes that are repressed at the level of transcription by v-*src*. From the repertoire of modulated genes, we selected 20 genes (10 induced and 10 repressed) that were robustly changed by v-*src* in the screen of Affymetrix DNA arrays. We then validated and quantified the transcriptional changes of most of the 20 selected genes by real-time PCR using array-independent sets of primer oligos and new preparations of RNA. The function of several robustly induced and repressed genes sheds new light on the molecular mechanism of oncogenic transformation.

Results and discussion

Identification of v-src-modulated genes

Analysis of GeneChip® Chicken Genome Arrays probed with RSV-SR wild type and RSV-SR-td106 mutant derived probes revealed that circa 10% of transcripts were changed. From 28,418 chicken genes investigated in the screen, 17,868 were detected at least once in RSV-SR or RSV-SR-td106 mutant screens. Among the detected genes, 1602 were induced and 1386 were repressed in v-*src* transformed cells. These changes were considered significant as they had a *p*-value of 0.05 or less. When a filter of two moderately stringent criteria was applied to the data, namely that the changes had to be more than 2.5 fold (linear, in either direction) and the *p*-value had to be less than 0.05, the number of modulated transcripts decreased by more than 3 fold to 811. Among the selected transcripts, 409 were induced and 402 were repressed by v-*src* (see Figure A representing a heat map of hierarchical clustering and Table A listing all 811 modulated sequences in Supplementary data). This set of data contained several genes that had been previously documented as v-*src*-modulated genes: urokinase-type plasminogen activator, gene for glucose transporter protein, and nephroblastoma overexpressed gene (Leslie et al., 1990; White et al., 1991; Scholz et al., 1996).

Validating selected transcripts by RT-PCR

We chose 20 robustly modulated genes from the "stringent set" of 811 modulated transcripts for further analysis. Among these 20 genes, 10 were induced and 10 repressed (Table 1). We isolated new RNAs from independent RSV-SR transformed and RSV-SR-td106 infected CEFs and generated the array-independent set of primer oligos to validate and quantify the changes by another technique, real-time PCR (RT-PCR). Using a housekeeping transcript, GAPDH, as a control for quantification and documenting the quality of each of the amplified products by melting curve analysis, we confirmed most (17 out of 20) of the transcriptional changes identified in the array screen. The changes scored by the GeneChip® Chicken Genome Array screen were in most cases larger than the changes measured by RT-PCR, even when the same RNA was used for the assays. This is often the case because of the intrinsic difficulty in setting the right background for the array analysis, especially with low signals from un-induced transcripts (personal communication from T. Hunter).

Gene ontology analysis

In an effort to determine trends in the regulation of classes of genes in response to v-*src* transformation, lists of Affymetrix Probe Set IDs for 811 genes that are significantly increased or significantly decreased (Table A) were submitted separately to DAVID 2006 (http://niaid.abcc.ncifcrf.gov/home.jsp). DAVID is a program that facilitates the transition from genome-scale data sets to biological meaning (Dennis et al., 2003). The software identified 41 and 47 terms that are significantly over-represented in a functional annotation chart of the genes with increased or decreased expression, respectively. The most significantly over-represented terms for the genes with increased expression included many related to phosphatase activity, proto-oncogenes, nuclear proteins, and DNA binding. In contrast, the most significantly over-represented terms for the genes with decreased expression included several related to the cytoskeleton, extracellular matrix, cell adhesion, endoplasmic reticulum, signal peptides, and glycoproteins. The Probe Set IDs associated with each of these terms are presented in Supplementary Table B. The terms associated with down-regulated genes seem to connote normal cellular growth, attachment, and secretion of regulatory factors, while those associated with up-regulated genes hint at abnormal DNA replication. One stand-out feature is the number of phosphatases that are up-regulated in the transformed cells.

Integrating and interpreting the transcriptional fingerprint of v-src in CEFs

Two other approaches were elected to analyze the data globally, focusing on the signaling system. In the first approach, we used the most recent version (2.1) of GenMAPP (Gene MicroArray Pathway Profiler) and MAPPFinder, two software packages from Gladstone Institutes of the University of California at San Francisco (www.genmap.org) to analyze changes in the outcomes of signaling pathways caused by v-*src*-modulated genes. We uploaded 2988 transcripts that were changed by v-*src*, with *p*-values no higher than 0.05, into the GenMAPP program and viewed the data on pathway profiles (MAPPFinder) that represent major biological pathways and functional grouping of genes (Doniger et al., 2003). Several signaling pathways, including TGF beta, MAP kinase, cell cycle, and the kinin-kallikrein component of the complement activation scored well in the GenMAPP analysis in terms of