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Research Article

Long-term in vitro, cell-type-specific genome-wide reprogramming of gene expression

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Abstract

We demonstrate a cell extract-based, genome-wide and heritable reprogramming of gene expression in vitro. Kidney epithelial 293T cells have previously been shown to take on T cell properties following a brief treatment with an extract of Jurkat T cells. We show here that 293T cells exposed for 1 h to a Jurkat cell extract undergo genome-wide, target cell-type-specific and long-lasting transcriptional changes. Microarray analyses indicate that on any given week after extract treatment, ~2500 genes are upregulated >3-fold, of which ~900 are also expressed in Jurkat cells. Concomitantly, ~1500 genes are downregulated or repressed, of which ~500 are also downregulated in Jurkat cells. Gene expression changes persist for over 30 passages (~80 population doublings) in culture. Target cell-type specificity of these changes is shown by the lack of activation or repression of Jurkat-specific genes by extracts of 293T cells or carcinoma cells. Quantitative RT-PCR analysis confirms the long-term transcriptional activation of genes involved in key T cell functions. Additionally, growth of cells in suspended aggregates, expression of CD3 and CD28 T cell surface markers, and interleukin-2 secretion by 293T cells treated with extract of adult peripheral blood T cells illustrate a functional nuclear reprogramming. Therefore, target cell-type-specific and heritable changes in gene expression, and alterations in cell function, can be promoted by extracts derived from transformed cells as well as from adult primary cells.

Keywords: 293T cell; Cell extract; Gene expression; Jurkat cell; Microarray; Nuclear reprogramming

Introduction

Increasing evidence indicates that the differentiation program of a somatic nucleus is not necessarily permanently locked into a specific fate. Functional nuclear reprogramming has been demonstrated through various approaches, including transplantation of somatic nuclei into unfertilized oocytes of eggs [1–6], cell fusion [7–9] and incubation with lysates of undifferentiated cells such as eggs [10] or blastema cells [11]. Reprogramming of somatic cells has been shown to occur after transplantation into ectopic sites [12–14], upon ectopic expression of transcription factors in vivo [15], by disruption of intercellular signaling and by co-culture (reviewed in [16]).

A somatic cell-free system which promotes alterations of cell fate has recently been described [17,18]. The approach consists of incubating reversibly permeabilized cells of one type in a nuclear and cytoplasmic extract from a chosen 'target' cell type. This approach was used to show that kidney epithelial 293T cells permeabilized with streptolysin O (SLO) [19] and exposed to an extract of Jurkat T cells can take on T cell properties. The extract promoted nuclear uptake of transcription factors, expression of T cell antigens and establishment of a T-cell-specific signaling pathway [17]. Similar extracts of rat cardiomyocytes were also shown to induce expression of cardiomyocyte markers in adipose stem cells [20]. Additionally, induction of dedifferentiation has been reported by

Abbreviations: dNTP, deoxynucleotide triphosphate; HBSS, Hank's balanced salt solution; IL-2, interleukin-2; RT-PCR, reverse transcription PCR; SLO, streptolysin O.

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treatment of 293T cells or C2C12 cell-derived myotubes with extracts of *Xenopus* eggs [10] or newt regenerating limbs [11]. None of these studies, however, provide a genome-wide assessment of gene expression changes elicited by extract-based reprogramming.

Microarrays have proven valuable for investigating expression differences of a large number of genes, providing insights into the molecular biology of cell differentiation and disease. Oligonucleotide arrays allow simultaneous relative quantification of thousands of transcripts, facilitating identification of differentially expressed genes. Commercially available arrays require micrograms of RNA to produce quantifiable hybridization signals, but procedures have been developed to generate target templates from submicrogram amounts of RNA suitable for cDNA and oligonucleotide arrays [21–24]. The latter approach combines exponential and linear RNA template amplification using PCR and T7 RNA polymerase, and was validated by comparing hybridization signals with those obtained from conventional linear amplification and by quantitative reverse transcription (RT)-PCR [24].

Here, we show that a 1-h exposure to a 'target' cell extract elicits a heritable and genome-wide target cell-type-specific reprogramming of gene expression. We analyzed, in four independent experiments and over 12 weeks, the gene expression profile of 293T cells treated with a Jurkat extract, and compared this profile to that of 293T cells, 'target' Jurkat cells, culture controls, and 293T cells exposed to their own extract or to an irrelevant NCCIT teratocarcinoma cell extract. The results argue that onset and duration of global and gene-specific transcriptional changes are highly dynamic. Furthermore, reprogramming of 293T cells in extract of peripheral blood T cells indicates that specific transcriptional and functional modifications can be elicited by subcellular fractions of not only transformed cells but also primary adult cells.

Materials and methods

Cells

Jurkat cells (clone E6-1), human carcinoma NCCIT cells [25], and 293T cells were cultured in RPMI 1640 containing 10% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate and non-essential amino acids (complete RPMI). Extract-treated and control cells were seeded at 100,000 cells per well in a 48-well plate and cultured in 250 μl complete RMPI containing 50 U/ml recombinant interleukin-2 (IL-2) and magnetic beads bearing antibodies to CD3 and CD28 (Dynal Biotech; 3 beads/cell). This culture system was referred to as T cell growth conditions. Human T cells were purified from buffey coats pooled from healthy donors [26] and immediately used for extract preparation.

Cell extracts

Jurkat cells were harvested and concentrated to 50×10^6 cells/ml in complete RPMI. Cells were stimulated with 40 ng/ml anti-CD3 antibodies (clone SpvT3d; a gift from Dr. A.M. Rasmussen) and 0.1 μM phorbolmyristylacetate for 2 h at 37°C. Cells were washed in PBS and in cold cell lysis buffer (20 mM HEPES, pH 8.2, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol and protease inhibitors), sedimented at $400\times g$ and resuspended in 1 volume of cell lysis buffer. Cells were sonicated on ice in 250-μl aliquots using a Labsonic-M pulse sonicator and a 2-mm-diameter probe (B. Braun Biotech) until all cells and nuclei were lysed. The lysate was sedimented at $15{,}000\times g$ for 15 min at 4°C. The supernatant was aliquoted, frozen in liquid nitrogen and stored for up to 6 months at −80°C. Protein concentration of the Jurkat extract was 42.5 ± 4.6 mg/ml (Bradford), pH was 7.0 ± 0.0, and osmolarity was 391 ± 38 mosM (average of 9 batches). Extracts of T cells, NCCIT cells, and of 293T cells were prepared similarly but without stimulation.

Cell permeabilization

293T cells were washed twice in cold PBS and in cold Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS; Gibco-BRL). Cells were resuspended in aliquots of 100,000 cells/100 μl HBSS (or multiples thereof up to 500 μl), aliquots placed in 1.5-ml tubes and centrifuged at $120\times g$ for 5 min at 4°C in a swing-out rotor. Sedimented cells were suspended in 97.7 μl cold HBSS, tubes placed in an H_2O bath at 37°C for 2 min and 2.3 μl SLO (Sigma-Aldrich; 100 μg/ml stock diluted 1:10 in cold HBSS) was added to yield a final SLO concentration of 230 ng/ml. Samples were incubated horizontally for 50 min in an H_2O bath at 37°C with occasional agitation and placed on ice. SLO was diluted with 200 μl cold HBSS and cells were sedimented at $120\times g$ for 5 min at 4°C. Permeabilization was assessed by monitoring uptake of a 70,000 M_r Texas red-conjugated dextran (Molecular Probes; 50 μg/ml) in a separate sample 24 h after resealing and replating cells (data not shown).

Extract treatment and cell culture

Following permeabilization, 100,000 cells were suspended in 100 μl Jurkat, NCCIT, 293T or T cell extract, as indicated, containing an ATP-regenerating system (1 mM ATP, 10 mM creatine phosphate, 25 μg/ml creatine kinase; Sigma-Aldrich), 100 μM GTP (Sigma-Aldrich), and 1 mM of each NTP (Roche). Cells were incubated for 1 h at 37°C in an H_2O bath with occasional agitation. To reseal plasma membranes, the extract was diluted with complete RPMI containing 2 mM $CaCl_2$ and antibiotics. Cells were transferred to a 48-well plate at 100,000 cells per well. After 2 h, floating cells were removed and seeded cells were cultured