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Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes

Lucia Spangenberg[b,1], Patricia Shigunov[a,1], Ana Paula R. Abud[a], Axel R. Cofré[a], Marco A. Stimamiglio[a], Crisciele Kuligovski[a], Jaiesa Zych[a], Andressa V. Schittini[a], Alexandre Dias Tavares Costa[a], Carmen K. Rebelatto[c], Paulo R.S. Brofman[c], Samuel Goldenberg[a], Alejandro Correa[a], Hugo Naya[b], Bruno Dallagiovanna[a,*]

[a] *Instituto Carlos Chagas, Fiocruz-Paraná, Rua Professor Algacyr Munhoz Mader, 3775, Curitiba-PR 81350-010, Brazil*
[b] *Unidad de Bioinformática, Institut Pasteur Montevideo, Mataojo 2020, Montevideo 11400, Uruguay*
[c] *Núcleo de Tecnologia Celular, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição, 1155, Curitiba-PR 80215-901, Brazil*

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Abstract Adipocyte stem cells (hASCs) can proliferate and self-renew and, due to their multipotent nature, they can differentiate into several tissue-specific lineages, making them ideal candidates for use in cell therapy. Most attempts to determine the mRNA profile of self-renewing or differentiating stem cells have made use of total RNA for gene expression analysis. Several lines of evidence suggest that self-renewal and differentiation are also dependent on the control of protein synthesis by posttranscriptional mechanisms. We used adipogenic differentiation as a model, to investigate the extent to which posttranscriptional regulation controlled gene expression in hASCs. We focused on the initial steps of differentiation and isolated both the total mRNA fraction and the subpopulation of mRNAs associated with translating ribosomes. We observed that adipogenesis is committed in the first days of induction and three days appears as the minimum time of induction necessary for efficient differentiation. RNA-seq analysis showed that a significant percentage of regulated mRNAs were posttranscriptionally controlled. Part of this regulation involves massive changes in transcript untranslated regions (UTR) length, with differential extension/reduction of the 3′UTR after induction. A slight correlation can be observed between the expression levels of differentially expressed genes and the 3′UTR length. When we considered association to polysomes, this correlation values increased. Changes in the half lives were related to the extension of the 3′UTR, with longer UTRs mainly stabilizing the transcripts. Thus, changes in the length of these extensions may be associated with changes in the ability to associate with polysomes or in half-life.

* Corresponding author. Fax: +55 41 33163267.
E-mail address: brunod@tecpar.br (B. Dallagiovanna).
[1] Both authors contributed equally to this work.

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Introduction

Human adipose tissue-derived stromal cells (hASC) are readily isolated from the pools of cells resident in the vascular stroma of adipose tissue. ASCs proliferate and self-renew and, due to their multipotent nature, they can differentiate at least in vitro into several tissue-specific lineages, including the chondrogenic, osteogenic, adipogenic and miogenic lineages (De Ugarte et al., 2003; Gimble et al., 2007). Adipose tissue is ubiquitous and large quantities are easily accessible with minimal invasion procedures (Baer and Geiger, 2012). These characteristics make these cells ideal candidates for use in cell therapy. An understanding of the biological process committing the cell to differentiation into a specific cell type is essential for the successful repair of injured tissue.

Cytokines, growth factors and extracellular matrix components in the microenvironment determine stem cell fate, by regulating the switch from self-renewal to differentiation (Kratchmarova et al., 2005). However, the downstream effectors and the gene regulatory networks controlling these processes remain unclear. Gene expression profiling has provided insight into the molecular pathways involved in ASC self-renewal and differentiation (Ivanova et al., 2002; Song et al., 2006). Genome-wide analyses based on microarray hybridization and, more recently, next generation sequencing, have been carried out to assess the global expression of gene networks.

Most attempts to determine the mRNA profile of self-renewing or differentiating cells have made use of total RNA for hybridization to microarrays or RNA-Seq analysis (Jeong et al., 2007a; Menssen et al., 2011). High-throughput analyses in eukaryotes comparing mRNA and protein levels have indicated that there is no direct correlation between transcript levels and protein synthesis, suggesting a high degree of posttranscriptional regulation in eukaryote cells (Washburn et al., 2003; Keene, 2007; Tebaldi et al., 2012). This hampers the classical transcriptome-based approach to investigate controlled expression in differentiating cells. Protein abundance can be controlled and refined through the regulation of gene expression at various complementary levels. Several lines of evidence from different organisms suggest that stem cell self-renewal and differentiation are also dependent on the control of protein synthesis by posttranscriptional mechanisms (Keene, 2007; Sampath et al., 2008; Haston et al., 2009; Kolle et al., 2011). The analysis of the mRNA fraction associated to polysomes has been used as a strategy to analyze posttranscriptional mechanisms involved in the control of translation (Fromm-Dornieden et al., 2012). This posttranscriptional regulation is mediated by various molecules, such as microRNAs, noncoding RNAs and RNA binding proteins. *Trans*-acting factors recognize and bind sequences or structural elements, mostly in the untranslated regions (UTRs) of mRNAs (Mittal et al., 2009; Bar et al., 2008; Keene, 2010). Posttranscriptional control may be mediated by, amongst other things, modifications to mRNA stability or by the inhibition of transcript association with translating ribosomes.

We used adipogenic differentiation as a model, to investigate the extent to which posttranscriptional regulation controlled gene expression in hASCs. We focused on the initial steps of cell differentiation and isolated both the total mRNA fraction and the subpopulation of mRNAs associated with translating ribosomes. RNA-seq analysis showed that a significant percentage of regulated mRNAs were controlled both at the translational level and by changes to their half lives. Part of this regulation is associated with differential extension/reduction of the 3′UTR after induction.

Materials and methods

Isolation, culture, and differentiation of hASCs

Stem cells were obtained from adipose tissue from obese human donors (two males and one female, ages: 41, 52, 23). All samples were isolated, collected after informed consent had been obtained, in accordance with guidelines for research involving human subjects, and with the approval of the Ethics Committee of Fundação Oswaldo Cruz, Brazil (approval number 419/07). hASCs were isolated, cultured and characterized as previously described (Rebelatto et al., 2008). Briefly, 100 ml of adipose tissue was washed with sterile phosphate-buffered saline (PBS) (Gibco Invitrogen). A one-step digestion by 1 mg/ml collagenase type I (Invitrogen) was performed for 30 min at 37 °C during permanent shaking and was followed by filtration through first a 100- and then 40-μm mesh filter (BD FALCON, BD Biosciences Discovery Labware, Bedford, MA, USA). The cell suspension was centrifuged at 800 *g* for 10 min, and contaminating erythrocytes were removed by erythrocyte lysis buffer, pH 7.3. The cells were washed and then cultivated at a density of 1×10^5 cells/cm^2 in T75 culture flasks in DMEM-F12 (Gibco Invitrogen) supplemented with 10% FCS, penicillin (100 units/ml), and streptomycin (100 μg/ml). The medium was changed 2 days after the initial plating. The culture medium was then replaced twice each week. ASCs were subcultured after the cultures had reached 80% to 90% confluence; cells were detached by treatment with 0.25% trypsin/EDTA (Invitrogen) and were replated as passage-1 cells (the process was then continued). The characterization of the cells has been done following the minimal criteria for defining multipotent mesenchymal stromal cells as determined by the International Society for Cellular Therapy (Dominici et al., 2006). All tests were performed with cell cultures at passages 3 to 5. For adipogenic differentiation, hASCs were treated with hMSC Adipogenic Differentiation Bullet Kit (Lonza), in accordance with the manufacturer's instructions. Briefly, adipogenic differentiation was induced by 6 day cycles of induction/maintenance during 21 days. Induction medium contained the adipogenic inducers insulin, dexamethasone, indomethacin and IBMX; maintenance medium contained insulin. The medium was changed every 3 days. The degree of adipogenic differentiation was determined by assessing the cytoplasmic accumulation of triglycerides by staining with Oil Red O or Nile Red (Sigma-Aldrich), as described by Rebelatto et al. (2008). We also performed reverse transcription-polymerase chain reaction (RT-PCR) to estimate the amount of adipocyte-specific fatty acid-binding protein 4 (FABP4) mRNA. A list of the primers used is provided in Supporting Information Table S1.

Sucrose density gradient separation and RNA purification

Polysomal fractions were prepared with a modified version of the procedure described by Holetz et al. (2007). In brief,