SUPPLEMENTARY FILE 9

Materials and Methods

In-solution tryptic digestion. Equal amounts of nuclear proteins (30 µg) from upregulated (upBRG1) and silenced (siBRG1) BRG1 samples were lyophilized and resuspended in 100 µL of 0.1 M triethylammonium hydrogen carbonate (TEAB) buffer pH 8.0. Equal amounts of nuclear proteins (30 µg) were also processed from control samples for upBRG1 (CMV) and siBRG1 (CTRL) obtained as previously described [1, 2]. Bovine β-Lactoglobulin (LACβ, 1 µg) was spiked in each sample to serve as an internal standard for experimental bias correction. Proteins were reduced by adding 1 µL of 1% SDS and 2 µL of 50 mM tris (2-carboxyethyl) phosphine (TCEP) and heating at 60°C for 1 h. Free thiol groups of cysteine residues were alkylated by adding 1 µL of 400 mM iodoacetamide and incubating for 30 min at room temperature in the dark with gentle agitation. Proteins were then digested overnight at 37°C with trypsin in 0.1 M TEAB pH 8.0 (protein/trypsin ratio 50:1 w/w).

iTRAQ labeling and peptide fractionation by OFFGEL electrophoresis. The resulting peptides were tagged with the iTRAQ reagents Multiplex Kit (AB Sciex, Foster City, CA, USA). Each sample was labeled with one of the isobaric tags (116 for upBRG1, 117 for siBRG1, 114 for CMV, 115 for CTRL) reconstituted with 50 µL of isopropanol. The reaction was left to stand at room temperature for 60 min and then blocked by incubating with 8µL of hydroxylamine 5% for 15 min. The mixtures of labeled peptides were then pooled and dried under vacuum. The lyophilized peptides were dissolved in 800 µL of 5% CH_3CN/ 0.1% formic acid (FA), and loaded (2 x 400µL) onto C18 Macro SpinColumns (Harvard Apparatus). Elution was performed with 2 × 200 µL of 50% CH_3CN/ 0.1% FA. The samples were then dried under vacuum and dissolved in 360 µL of deionized water. A solution containing 6% glycerol and 0.3% IPG buffer pH 3-10 (Agilent, Santa Clara, CA, USA) was added to a final volume of 1.8 mL. Peptides were fractionated according to their pI on an Agilent 3100 OFFGEL fractionator using commercial 12 cm IPG pH 3-10 linear strips (GE Healthcare, Waukesha, WI, USA). The strips were rehydrated with 20 µL of rehydration solution (4.8% glycerol, 0.24% IPG buffer pH 3-10) per well. After a 30 min incubation, 150 µL of the sample solution were loaded per well. The isoelectric focalization was carried out at 20°C until a total voltage of 20 kV/h with a maximum current of 50 µA and a maximum power of 200 mW. After the focalization, peptide fractions (12/ for each group) were recovered in separate tubes and pH values were measured to check for the efficiency of the pH gradient. Fractions were then dried under vacuum, dissolved in 300 µL of 5% CH_3CN/ 0.1% FA, and loaded (2 x 150 µL) onto C18 Micro SpinColumns (Harvard Apparatus). Elution was performed with 2 × 100 µL of 50% CH_3CN/ 0.1% FA and eluted fractions were dried under vacuum and stored at -20 °C until MS analysis.

Liquid chromatography-tandem mass spectrometry. Lyophilized peptides obtained from OFFGEL fractionation were dissolved in 8 µL of 5% CH_3CN/ 0.1% FA; 5 µL of the resulting sample were injected for LC-MS/MS analysis. MS analysis was performed on a LTQ Orbitrap Velos Pro from Thermo Electron (San Jose, CA) equipped with a NanoAcquity UPLC system from Waters (Milford, MA, USA) [3, 4]. Peptides were trapped on a home-made (5 µm 200 Å Magic C18 AQ 0.1 × 2 mm) pre-column (Michrom, Auburn, CA, USA) and separated on a home-made (5 µm 100 Å Magic C18 AQ, 0.75 × 15 mm) column (Michrom). The analytical separation was run for 65 min using a gradient of 99.9% H_2O/ 0.1% FA (solvent A) and 99.9% CH_3CN/ 0.1% FA (solvent B). The gradient was run as follows: 0–1 min 95% A and 5% B, then to 65% A and 35% B at 55 min, and 20% A and 80% B at 65 min at a flow rate of 220 nL/min. For MS survey scans, the OT resolution was set to 60000 and the ion population was set to 5 × 105 with an m/z window from 400 to 2000. A maximum of 3 precursors was selected for both the collision-induced dissociation (CID) in LTQ and the high-energy C-trap dissociation (HCD) with analysis in the OT. For MS/MS in the LTQ, the ion population was set to 7 x 103 (isolation width of 2 m/z) while for MS/MS detection in the OT, it was set to 2 × 105 (isolation width of 2.5 m/z), with resolution of 7500, first mass at m/z = 100, and maximum injection time of 750 ms. The normalized collision energies were set to 35% for CID and 60% for HCD.

Data extraction, database interrogation and relative protein quantification. Peak lists were generated from raw data using the embedded software from the instrument vendor (extract_MSN.exe v5.0). After peaklist generation, the CID and HCD spectra were merged for simultaneous identification and quantification by using EasyProtConv [5]. The merged mgf files, combined from the 12 analyzed OFFGEL fractions, were used for protein identification and quantification with EasyProt software platform v2.2 [5]. For protein identification, parameters were specified as follows: database = uniprot_sprot (2011_02 of 08-Feb-2011); taxonomy = *Homo Sapiens*; precursor error tolerance = 25 ppm; variable modification = oxidized methionine; fixed modifications = carbamidomethylated cysteine, iTRAQ-labeled amino terminus and lysine; enzyme = trypsin; potential missed cleavage = 1; cleavage mode = normal; search round = 1, scoring model = CID_LTQ_scan_LTQ; instrument type = ESI-LTQ-Orbitrap. Protein and peptide scores were set up to maintain the false positive peptide ratio below 5%. For protein quantification, the isotopic correction was applied to reporter intensities according to the iTRAQ reagents certificate of analysis. iTRAQ reporter peak

intensities were further normalized using the spiked LACβ standard. For each protein, the mean, the standard deviation, and the coefficient of variation (CV) of relative peptide intensities were obtained for the two experimental groups by using the EasyProt Mascat quantification module that computes a per-peptide ratio from the reporter ion abundance values for the given peptide [5]. The ratio of a protein is then computed as the geometric mean of all peptide ratios belonging to the protein. A Student's t-test distribution, with a null hypothesis stating that the $\log_2$ of the protein ratio is equal to zero (confidence interval=95%) was computed by the algorithm. Proteins featuring a ratio outside the interval of confidence, excluding zero, were considered as differentially expressed in upBRG1 *versus* CMV and siBRG1 *versus* CTRL samples.

Bioinformatics analyses. An assessment of significantly enriched biological processes was performed according to gene ontology annotations by using the DAVID software v6.7 (Database for Annotation, Visualization and Integrated Discovery) [6, 7] by applying the Fisher exact test (EASE Score Threshold 0.001). The heat map representing the functional classification of up- and down-regulated proteins was constructed by clustering significant functional terms according to the DAVID enriched biological processes by using the TIBCO Spotfire software (Somerville, MA, USA).

References

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1. Alessio N, Squillaro T, Cipollaro M, Bagella L, Giordano A, Galderisi U. The BRG1 ATPase of chromatin remodeling complexes is involved in modulation of mesenchymal stem cell senescence through RB-P53 pathways. Oncogene. Oct 7 2010;29(40):5452-5463.

2. Napolitano MA, Cipollaro M, Cascino A, Melone MA, Giordano A, Galderisi U. Brg1 chromatin remodeling factor is involved in cell growth arrest, apoptosis and senescence of rat mesenchymal stem cells. J Cell Sci. Aug 15 2007;120(Pt 16):2904-2911.

3. Farina A, D'Aniello C, Severino V, et al. Temporal proteomic profiling of embryonic stem cell secretome during cardiac and neural differentiation. Proteomics. Oct 2011;11(20):3972-3982.

4. Severino V, Farina A, Colucci-D'Amato L, et al. Secretome profiling of differentiated neural mes-c-myc A1 cell line endowed with stem cell properties. Biochim Biophys Acta. Nov 2013;1834(11):2385-2395.

5. Gluck F, Hoogland C, Antinori P, et al. EasyProt--an easy-to-use graphical platform for proteomics data analysis. J Proteomics. Feb 21 2013;79:146-160.

6. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44-57.

7. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. Jan 2009;37(1):1-13.

8. Angelov D, Molla A, Perche PY, et al. The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. Mol Cell. Apr 2003;11(4):1033-1041.

9. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. FACT facilitates transcription-dependent nucleosome alteration. Science. Aug 22 2003;301(5636):1090-1093.

10. Chakravarti D, Hong R. SET-ting the stage for life and death. Cell. Mar 7 2003;112(5):589-591.

11. de Las Heras JI, Batrakou DG, Schirmer EC. Cancer biology and the nuclear envelope: a convoluted relationship. Semin Cancer Biol. Apr 2013;23(2):125-137.

12. De Vos WH, Houben F, Hoebe RA, et al. Increased plasticity of the nuclear envelope and hypermobility of telomeres due to the loss of A-type lamins. Biochim Biophys Acta. Apr 2010;1800(4):448-458.

13. Dechat T, Pfleghaar K, Sengupta K, et al. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev. Apr 1 2008;22(7):832-853.

14. Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL. The nuclear lamina comes of age. Nat Rev Mol Cell Biol. Jan 2005;6(1):21-31.

15. Huang JY, Chen WH, Chang YL, Wang HT, Chuang WT, Lee SC. Modulation of nucleosome-binding activity of FACT by poly(ADP-ribosyl)ation. Nucleic Acids Res. 2006;34(8):2398-2407.

16. Kamakaka RT, Biggins S. Histone variants: deviants? Genes Dev. Feb 1 2005;19(3):295-310.

17. Ragnauth CD, Warren DT, Liu Y, et al. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation. May 25 2010;121(20):2200-2210.

18. Sarma K, Reinberg D. Histone variants meet their match. Nat Rev Mol Cell Biol. Feb 2005;6(2):139-149.

19. Severino V, Malorni L, Cicatiello AE, et al. An integrated approach based on multiplexed protein array and iTRAQ labeling for in-depth identification of pathways associated to IVF outcome. PLoS One. 2013;8(10):e77303.
20. Winkler DD, Muthurajan UM, Hieb AR, Luger K. Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. J Biol Chem. Dec 2 2011;286(48):41883-41892.
21. Zhang R, Poustovoitov MV, Ye X, et al. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell. Jan 2005;8(1):19-30.