Quantitative Proteome Analysis of Telomere-differential Human Breast Epithelial Cells

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Regulation of telomeres is pivotal to replicative lifespan in many types of eukaryotic cells. In normal cells, telomeres are shortened with each cell cycle, limiting the number of times a cell can divide. In contrast, telomere length can be maintained in cancer cells or immortalized cell lines by telomerase or an alternative lengthening of telomeres (ALT) mechanism. An understanding of the molecular mechanism of telomerase activation or ALT will facilitate development of telomere- and telomerase-based therapeutics for cancers. Isotope coded affinity tags (ICAT) was used in combination with electrospray ionization ion trap mass spectrometry (MS) to examine the relative protein abundances in telomere length-differential human breast epithelial cells transfected with human TERT gene (hTERT). The analysis resulted in the quantitation of thousands of proteins between epithelial cells with short and long telomeres.

Human breast epithelial cells from different patients were transfected with hTERT gene to establish the 184-hTERT and 90p-hTERT cells. Each type of cells were harvested with short telomeres at low population doublings levels (PDLs) and with long telomeres at high PDLs. Proteins extracted from 184-hTERT and 90p-hTERT cells with short and long telomeres were labeled with the light and heavy isotopic ICAT reagents, respectively. riefly, proteins from either sample were dissolved in 80 µL of denaturing buffer containing 6 M guanidine hydrochloride and 50 mM NH_4HCO_3, pH 8.3. Each sample was reduced by adding 1µL of 100 mM TCEP·HCl and boiling them in a water bath for 10 min. The reduced samples were transferred to each vial containing light or heavy ICAT reagent that was already dissolved in 20 µL of acetonitrile. The reaction mixtures were then incubated at 37 °C for 2 h. After the ICAT labeling of the Cys residues, the two samples were combined, desalted into 50 mM NH_4HCO_3, pH 8.3, and digested with trypsin (Promega, Madison, WI) overnight at 37 °C in an enzyme to protein ratio of 1:50 (w/w). To quench the trypsin activity, the digested sample was incubated in a boiling water bath for 10 min, and phenylmethanesulfonyl fluoride was added to a final concentration of 1 mM. The ICAT labeled peptides were isolated by avidin chromatography and fractionated by strong cation exchange (SCX) chromatography. Ten-cm µLC-electrospray columns were coupled online with an ion-trap mass spectrometer (LCQ Deca XP, Thermo Finnigan, San Jose, CA) to analyze cleaned and uncleaned ICAT-labeled BSA peptides. To construct the µLC-electrospray columns, fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) with 75 µm i.d. were flame-pulled to construct a 10-cm fine i.d. tip (i.e., 5-7 µm) against which reversed-phase particles of Luna C18(2) (3-µm diameter, 100-Å pore size) or Jupiter C18 (5-µm pore size, 300-Å pore size) (Phenomenex, Torrence, CA) were slurry packed using a slurry packing pump (Model 1666, Alltech Associates, Deerfield, IL). The packed µLC-electrospray columns were connected via a stainless union to an Agilent 1100 capillary LC system (Agilent Technologies, Paolo Alto, CA), which delivers solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). After loading 5 µL of sample, the ICAT-labeled peptides were eluted at a flow rate of 300 nL/min under the gradient of 0% (or 2%)-45% for 70 min and 45%-85% for 10 min. The ion trap mass spectrometer was operated in a data-dependent MS/MS mode in which three most intense peptide molecular ions in the MS scan were sequentially and dynamically selected for subsequent collision-induced dissociation (CID) with normalized collision energy of 35%. The MS spectrum for the molecular ions was acquired with 2 µscans at the mass range of *m/z* 300-2000, and the CID spectrum for the fragment ions was acquired with 3 µscans. The voltage and

temperature for the capillary of the ion source was 10 V and 180 °C, respectively. Peptides were identified using TurboSEQUEST and quantified with XPRESS (ThermoFinnigan, San Jose, CA).

In the comparison of the relative abundances of the 184-hTERT cells with short and long telomeres, more than 8800 unique ICAT-labeled peptides corresponding to over 3000 proteins were quantified. The analysis of the 90p-hTERT cells with long and short telomeres resulted in the relative quantitation of more than 3300 unique proteins from almost 11000 unique ICAT-labeled peptides. Proteins from nearly every molecular function, biological process and subcellular compartment were identified and quantitated. Although most of the proteins are not significantly changed in their expression, there are several hundred proteins exhibiting medium changes (1.7-3.0 fold) in relative abundance between cells with short and long telomeres. In addition, more than 200 proteins showed an extreme changes (above 3 fold) in relative abundance. The overlap between proteins found in the global analysis of the different cell types was 73% at the protein level and 68% at the peptide level. A significant overlap in the unique proteins and peptides identified in the ICAT analysis of the BE-184 and BE-90 cells with differing telomere length was observed, revealing the usefulness of comparing protein abundances obtained from different cell types using this solution-based technology.

One of the future goals of proteomics is to compare protein abundances from different cell systems, and what affect specific treatments have on these measurements. Obviously the first need for comparative quantitative proteomics is to have a significant overlap in the proteins identified in the different analyses. For 2D-PAGE-based studies, the overlap is based on the number and position of the protein spots that are visualized on the gel. Obviously this type of visualization allows protein overlap of two different proteome samples to be readily discerned, however, such is not the case for solution-based methods for quantitative proteomics. While methods such as ICAT hold many advantages over traditional 2D-PAGE-based methods, there are no studies that provide a measure of the overlap of proteins identified when such techniques are applied in inter-comparative analyses. In this study, epithelial cells obtained from two different human sources were cultured under conditions that result in a population of cells with short telomeres and a comparable population with long telomeres. After ICAT-labeling and multidimensional fraction followed by tandem MS identification of the labeled peptides, the number of unique peptides identified in the analysis of the BE-184 cell strain was compared to those identified in the analysis of the BE-90 cell strain. Of the over 3000 proteins identified in each of the two studies, 73% were found to be common to both. This level of overlap shows the utility of using a solution-based approach to perform quantitative proteomics and enables effective inter-comparisons of quantitative proteomic studies.

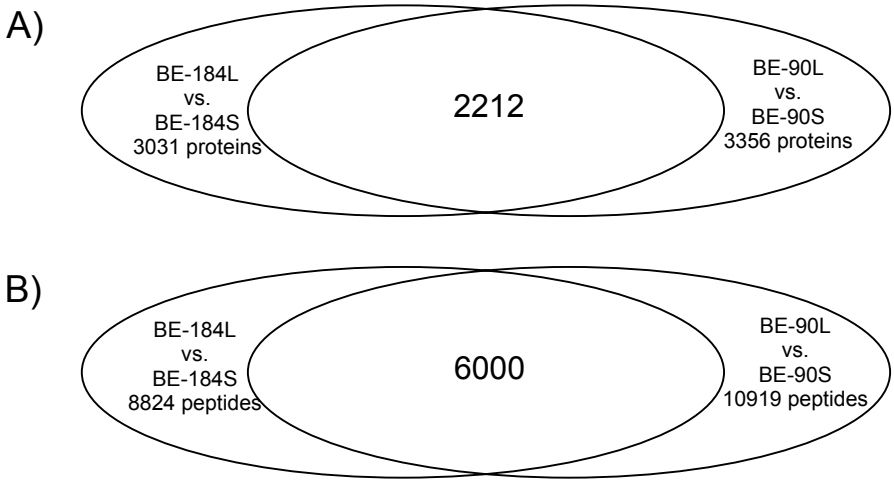

Figure 1. Venn diagram showing number of (A) proteins and (B) peptides that were common between the ICAT comparative analysis of breast epithelial cells with short and long telomeres extracted from two distinct patients (i.e. BE-90 and BE-184).