Summary:
Liquid chromatography (LC)-tandem mass spectrometry (MS/MS), especially multi-dimensional LC (2DLC)-MS/MS, is suitable for the large-scale determination of protein expression in cells to study proteomes. A shotgun approach, in which protein identification is achieved by separating and analyzing enzymatic digests without isolating individual proteins, enables us to analyze a mixture of proteins regardless of their physicochemical characteristics such as $M_r$, $pI$ and solubility. By applying this technology to profiling proteins expressed in mouse embryonic stem (ES) cells, we cataloged 1,790 proteins including 365 potential nuclear and 260 membrane proteins from whole cell lysates. In addition to many housekeeping proteins found in common with other cell types, the subset contained a group of regulatory proteins that may determine unique ES cell functions. To gain further insight into ES cell signaling, we focused on the plasma membrane and developed a method for the selective identification of cell surface proteins based on a combination of cell surface labeling and 2D LC-MS/MS technology. In total, 324 proteins including 235 proteins predicted to have signal sequences and/or transmembrane segments were identified from the ES cell surface. Besides known cell surface markers such as alkaline phosphatase, we identified 50 cluster of differentiation (CD) antigens and more than 80 cell signaling molecules including receptors, cell adhesion molecules and extracellular matrix proteins. Overall, these analyses revealed a number of potential signaling pathways known to function in ES cells, particular types of cells or most cell types, implying that the sheer diversity of expressed proteins may be one characteristic of ES cells. Although confirmation and extensive analyses are required to determine molecular functions and cellular roles of identified proteins, a catalog of expressed proteins is useful for surveying the characteristics of ES cells and/or searching for candidate molecular markers.