CONCLUSIONS

1) In this study, we have identified 7,797 protein groups in Jurkat T cells (according to Mascot algorithm), representing 7,733 unique proteins without considering isoforms. 254 of these proteins are encoded by 343 Chr16 genes, representing almost 50% of the Chr16 protein-coding genes expressed in Jurkat cells.

2) The best proteome coverage was reached using the CHAPS/Urea cell lysis and the RP-MC/MS workflow. Subcellular localization of both total and Chr16 proteins however revealed that membrane proteins are overrepresented in our approach, suggesting that optimization of sample preparation and fractionation procedures are required if we want to gain full coverage of hydrophobic and low abundance proteins.

3) MS analysis of the Jurkat secretome showed that some of the proteins identified in global lysates are potentially secreted and have been related to cancer (CAPs), highlighting their potential utility as cancer biomarkers. However, fewer secreted proteins have been detected. This suggests the application of additional growth conditions to stimulate the secretion of more proteins and complete our knowledge about Chr16 proteins.

4) This information constitutes the data source to set up targeted analyses (SRM and pseudo-SRM) for a more comprehensive subcellular view of Chr16 proteins, especially those annotated as CAPs. These methods will be employed for routine measurement of Chr16 proteins in different cell lines or tissues and, importantly, will be translated to disease-related studies.

INTRODUCTION

As part of the Chr16 (Spanish Consortium), our group has designed a pilot study to generate a comprehensive map based on high resolution data-dependent mass spectrometry to define the proteome coverage of the Chr16. This chromosome is estimated to encode 2,348 genes, far reported (Ensembl v70), with 886 protein-coding genes and we estimate that 75% of these protein-coding genes are expressed in lymphoid tissues.

We report here deep proteome analysis of the human T lymphoblast cell line (Jurkat cells). We compared different protein extraction methods and preparation conditions to gain coverage of all subsets of proteins (including membrane and secreted proteins).

Our results show that the combination of various workflows is critical to complement our current view of the human proteome. An important pillar that however remains to be solved is the coverage of integral membrane proteins, which are poorly represented in standard shotgun workflows.


4 Swiss Human Proteome (SpHPP) Project.