Identification of ZBRK1 homologue in mouse by a proteomic approach

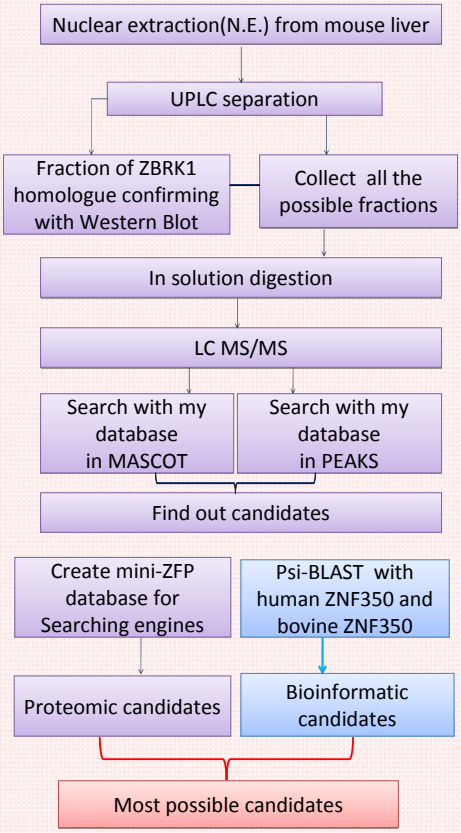
Miao-Hua Chang[1], Ju-Ming Wang[2], Shu-Hui Chen[1,3]*

[1] *Institute of Bioinformatics, National Cheng Kung University, Tainan, Taiwan*
[2] *Institute of Bioinformatics and Biosignal Transduction, National Cheng Kung University, Tainan, Taiwan*
*[3] *Department of Chemistry, National Cheng Kung University, Tainan, Taiwan*

Introduction

A novel human gene, ZBRK1, encodes a 58 kDa protein with an N-terminal KRAB(Kruppel-associated box) domain and eight central zinc fingers. The ZBRK1, also called as ZNF350, is known to interact with BRCA1 and induce its downstream genes. The evolutional gene addition and deletion make it hard to identify homologue protein between species. Until now, only bovine zinc finger protein 350 has been identified as human ZBRK1 homologue protein with their high similarity of their full sequences. In mouse, we failed to identify a homologue protein using previous strategy of highly similar amino acids sequences. Therefore, we develop a different strategy combined with ultra performance liquid chromatography (UPLC), mass spectrometry and bioinformatics in order to successfully overcome the genetic evolutional challenges and identify a homologue protein in mouse.

Methods



Results

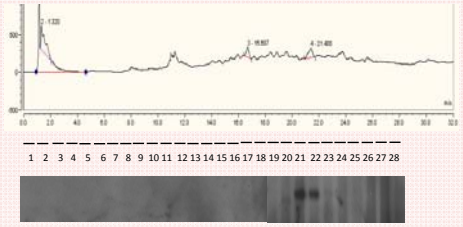
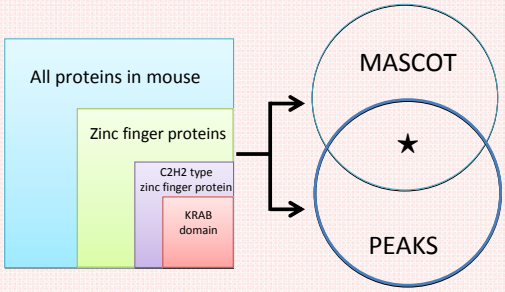


Figure 1. Separation condition of UPLC was optimized and confirmed with western blotting.



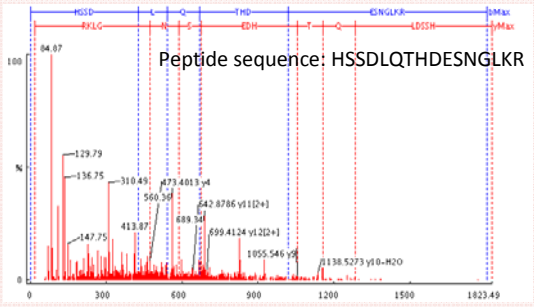


Figure 2. We create a minimal database based on the characteristics of ZBRK1, which contains mouse C2H2-type zinc finger proteins with KRAB domain. This database was used on both searching engines—PEAKS and MASCOT and the MS/MS result is shown above.

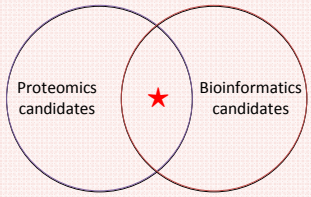


Figure 3. Both proteomics and bioinformatics (psi-BLAST) methods were used and the overlapping proteins are the most possible ZBRK1 homologue.

Conclusions

Considering about genetic addition and deletion, it is difficult to find out homologue by using gene alignments. In this study, we try to purify and enrich the homologue proteins. This helps to increase the signal and score of LC MS/MS protein identification. Building a characteristic minimal database not only filter the redundant proteins but rapidly process the most possible candidates. Bioinformatics (psi-BLAST) was also used to confirm our results. Our preliminary data shows that this is a potential promising strategy to identify a novel homologue protein.

Acknowledgements

This work was supported under a multidisciplinary project (NSC 96-2113-M-006 -013-MY3) granted by National Science Council in Taiwan. Especially thanks for Brian Munro 's very favors in using PEAKS studio5.1 and proteomic discussion.