Results

We observed precipitation in the separation chamber when the total cell extract from HeLa cells was separated in the IEF-FFE mode even when a detergent was included in the separation media. Application of the IZ-FFE mode significantly increased protein solubility. Separation of the total cell extract was performed in the IZ-FFE mode using cleavable detergent at high loading rates without precipitation of proteins in the separation chamber. This was also the case for the membrane protein extract of the HeLa cells. The separation media containing the cleavable detergent facilitated the coupling to LC-MS/MS since no cumbersome detergent removal was necessary. The identified membrane proteins from the LC-MS/MS analysis of the individual FFE fractions were analyzed based on the number of transmembrane domains using the TMHMM software (www.cbs.dtu.dk/services/TMHMM/).

Conclusions

FFE provides an alternative method for the separation of membrane proteins. Protein precipitation can be avoided by using the IZ-FFE mode. Coupling to LC-MS/MS is facilitated by the introduction of a cleavable detergent in the separation media.

Systematic analysis of protein interactions with tetraspanins by high-troughput, second generation proteomics technics

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Introduction

The tetraspanin superfamily of transmembrane proteins are clustered in compact structural groups forming specialized membrane microdomains (Tetraspanin Enriched Microdomains, TEM or TERM). Through heterolog and homolog interactions, tetraspanins regulate signalling processes mediated by cellular adhesion molecules, growth factor receptors and costimulatory proteins (Hemler, 2005). In spite of the growing interest for these proteins, the cytosolic interactions by which tetraspanins are involved in various receptor activation pathways, their cytoskeleton anchorage and in general the protein ligands that interact with these proteins are poorly known. In this work we have made a systematic analysis of interacting partners of different proteins that are presented in TERMs, including ICAM-1, VCAM-1, CD81, CD151 and EWI-2 (Barreiro and Yañez-Mo et al., 2005). This was accomplished by “pull-down” techniques and high-throughput protein identification of the captured ligands by mass spectrometry.

Material and methods

The schematic workflow is represented in Figure 1. Synthetic biotinylated peptides spanning the C-terminal cytoplasmic end of these proteins were incubated with extracts from different cell models, including HeLa cells and lymphocytes, and then captured using Streptavidin-sepharose microbeads. Proteins interacting with the peptides were subjected to digestion and the resulting peptides systematically analyzed by HPLC-linear ion trap MS/MS mass spectrometry. Proteins were automatically identified from the MS/MS spectra in a human database by using the pRatio software developed by our group (Navarro et al., 2007); error rate of protein identification was controlled by using decoy databases and was inferior to 5% (López-Ferrer et al., 2004).

Results

More than one hundred proteins from HeLa cells and a similar number from resting or CD3-activated

lymphocytes were identified interacting with any of the target peptides but not with the controls. These included membrane and cytosolic proteins, and also cytoskeleton proteins, like α-actinin and Filamin, which could play a physiologically relevant role in the function of tetraspanins. Interactions of tetraspanins with some of the most interesting ligands were validated by Western blotting and immunoprecipitation approaches.

Conclusions

Our results are contributing to improve our knowledge of molecular mechanisms underlying the biological role of TERM proteins and demonstrate the performance of second generation proteomic techniques for the systematic study of protein-protein interactions.

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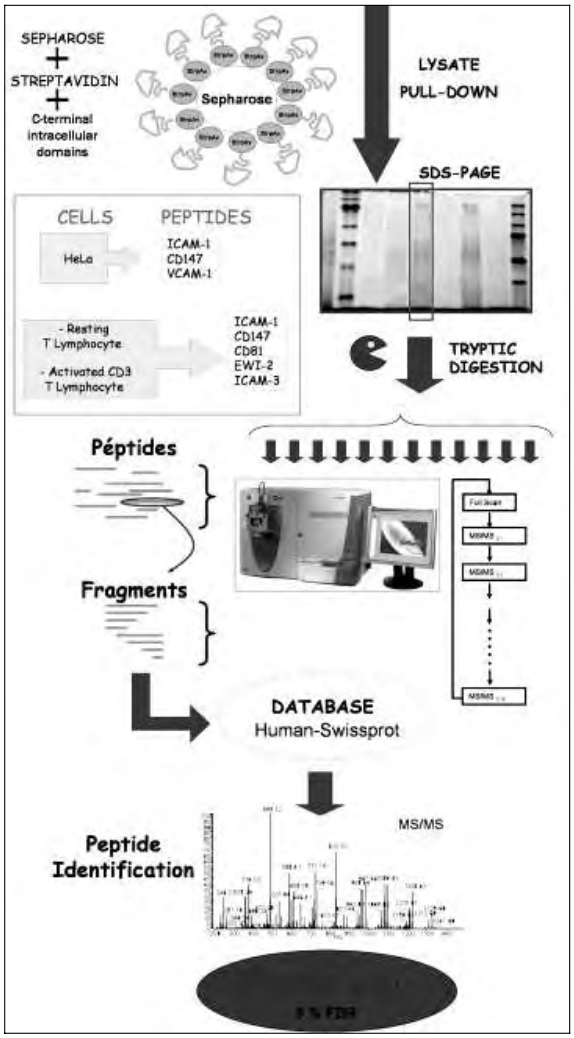

Figure 1: *Schematic design of the protocol used to identify tetraspanin-binding factors.*

Snake venomics of bitis species reveals large intragenus venom toxin composition variation. Application to taxonomy of congeneric taxa

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Venoms represent the critical evolutionary innovation that allowed advanced snakes to transition from a mechanical (constriction) to a chemical (venom) means of subduing and digesting prey larger than themselves. Venoms retain information on their evolutionary history, and are of potential taxonomical value. The protein composition of the venoms of the West African Gaboon viper (*Bitis gabonica rhinoceros*), the rhinoceros viper (*Bitis nasicornis*), and the horned puff adder (*Bitis caudalis*) were analyzed by RP-HPLC, N-terminal sequencing, SDS-PAGE, MALDI-TOF peptide mass fingerprinting, and CID-