Development of a new and sensitive detection technology for galectin cancer proteins

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Introduction

Detection of specific protein levels in tissue samples provides valuable biological information for prognosis and diagnosis. However, current proteomic tools give conflicting data because protein or mRNA abundance is measured rather than activity. In contrast, we have developed a protein detection assay using PamChip® microarray technology1,2,3,4 that visualizes functionally active proteins through a general fluorescent ligand.

As proof of concept we developed a detection assay for galectins. Galectins bind galactosides and are involved in cancer. In this assay immobilized specific antibodies capture the target protein from a cell lysate. After the unbound proteins are removed by a washing step, the presence of galectin is visualized by binding of a galactose-based fluorescent ligand. (Fig. 1)

PamChip® Functional Protein Assay

A Cell lysate
Protein of interest with fluorescent ligand

B Cell lysate
Protein of interest with fluorescent ligand

PamChip® microarray with immobilized antibodies against proteins of interest

Fig. 1. A. Schematic representation of protein detection technology with a fluorescent ligand. B. PamChip® protein/peptide/carbohydrate technology used.

Results

The galectin antibodies were immobilized by the Fc-binding protein A/G. The general ligand for galactoside was designed from the proteins bovine serum albumin (BSA) or asialofetuin (ASF) as multivalent scaffolds that were functionalized with lactose or lactosamine respectively. The synthesis of lactoside for BSA functionalization is described in scheme 1.

Scheme 1 Reaction conditions for the synthesis of lactoside carbamate: a) 3-bromo-1-propanol, iPr, CH3CN; b) NaN3, DMF, 100°C; c) H2, Pd/C, Boc2O, EIOAc; d) TFA/CH2Cl2, NaOMe, MeOH

ASF was enzymatically functionalized with lactosamine. The ligands were additionally derivatized with biotin to be visualized with anti-biotin antibodies to increase fluorescent signal necessary to obtain the best signal-to-noise ratio. (Fig. 2)

The detection method was used to visualize galectin-1 in a spiked E. coli cell lysate, which showed fluorescence corresponding to the amount of galectin-1. Finally, the cancer cell lines B16F10, HeLa and patient-derived lung tumor tissue (NSCLC) were tested and the latter two were found positive with galectin-1 which was verified by Western Blot analysis. (Fig. 3)

Fig. 2. Developed detection strategy: anti-galectin antibodies were immobilized on the microarray through protein A/G. Subsequently, galectin was captured from the cell lysate and bound by the general ligand for galacto, which was then visualized by an anti-biotin-antibody labeled with Cy3.

Fig. 3. A) Fluorescent detection of different amounts of galectin-1 spiked in 8 µg E. coli cell lysates. B) Detection of galectin-1 in HeLa and tumor (NSCLC) lysates. B16F10 and E. coli (negative control) cell lysates showed no fluorescence. C) Western Blot analysis was in accordance with the microarray results.

Conclusions

This poster describes a novel and sensitive detection technology for proteins based on their ligand binding capacity. With this method a detection limit of 9 ng galectin in cell lysates was obtained. The detection technology allows multiple protein levels to be measured in a single experiment to increase the comparability of the binding results. Together with the high sensitivity and the ease of performance the PamChip® protein detection technology appears very promising for use in a clinical setting.1

References


Acknowledgements

The authors would like to thank Linda Quarles van Ufford and Pieter Vader for cell culture, Gwenn Mulder for MALDI-TOF analysis, Johan Kemink for HSQC NMR, Iskandar Gandasasmita for the preparation of mechanically spotted protein A/G PamChips® and Nathaniel Martin for useful discussions.

Collaboration

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