Development of a MUC1-based reference bioassay for a novel *in vitro* breast-cancer detection system

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Abstract

Breast cancer is with more than 1.3 million new cases per year the most common type of cancer in women worldwide. In contrast, men are only less than 1% affected by this disease. In addition to the generally used methods of breast-cancer diagnosis like palpation, mammography, ultrasound, magnetic resonance imaging and biopsy, there are only a few tools available which indicate (early) breast cancer on the molecular level of the disease like Theros H/ISM and MGISM (bioTheranostics), Mammaprint™ (Agendia) or Onco*type* DX® (GenomicHealth).

The work presented here is part of the Interreg IV-A funded project MICROBIOMED (MICROtechnologies for BIOMEDical applications) and aims to develop a novel antibody-based in vitro breast-cancer detection system. Therefore we identified several breast-cancer tumor markers described in the literature for the set-up of an enzyme-linked immunosorbent assay (ELISA) based reference system. Additionally the Institute of Pathology of the University Hospital Aachen is searching for new breast-cancer specific biomarkers.

In this project, the tumor-marker MUC1 was used to establish a first proof-of-principle bioassay and a specific detection of cellular (breast) cancer specific MUC1 epitopes is presented. Finally the design will be transferred to the different biochip-platforms provided by the engineering partners.

Goal of the study

The goal of the project is the development of a new antibody-based breast-cancer detection system. To develop and establish the ELISA-based bioassay, MUC1, a proteoglycan with an extensive O-linked glycosylation of its extracellular domain, has been chosen. Therefore cells expressing MUC1 with different glycosylation-patterns as well as antibodies were used to set-up the ELISA-based reference system.

A MUC1 underglycosylation is associated with breast cancer and other carcinomas and can be utilized to discriminate between cancer and non-cancer cells [1]. Additionally, the cell surface protein can loose its apical distribution and be secreted into the circulation [2].

Two antibodies directed against two different MUC1-variants previously confirmed by flow cytometry were provided by the clinical partners from Maastricht University Hospital [3].

The monoclonal antibody (mAb) 214D4 recognizes human MUC1 independently of its glycosylation whereas mAb 5E5 recognizes a breast-cancer specific MUC1 glycosylation pattern - called Tn antigen [3]. As our clinical partners showed the specific binding in a cellular assay (FACS), now it would be beneficial to establish a standard ELISA-based assay using these antibodies and Tn as antigen.

Experimental design

The treatment of Chinese Hamster Ovary cell line with an O-glycosylation-deficiency (CHO-*ldl*D) with *N*-Acetylgalactosamine (GalNAc) leads to expression of the Tn antigen and binding of 5E5 is possible, whereas the untreated surface protein can only be detected via mAb 214D4. These Chinese Hamster Ovary cell line was stable transfected with a MUC1-F plasmid (CHO-*ldl*D Muc1) and used for ELISA.

Results

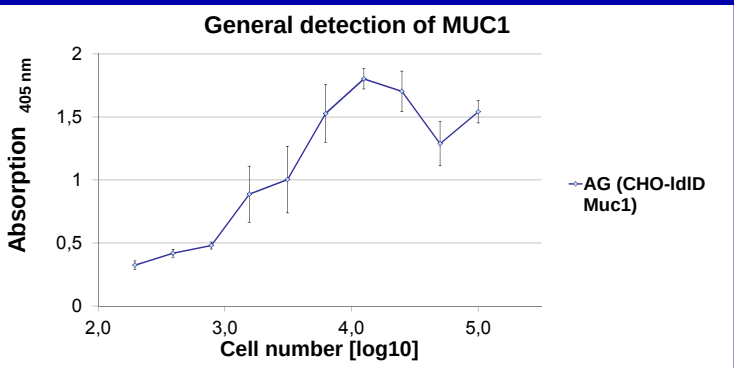

Fig.1: General detection of MUC1 on cells. The titration of cells showed that a medium absorption could be measured when using approximately 1x10^3 cells/well. To set-up the reference system, a sandwich-ELISA with CHO-*ldl*D Muc1 cells as antigen was performed. The mAb 214D4 was used for coating (1µg/well) and in a biotinylated form for the detection (21ng). This analysis is representative of three independent experiments.

[1] van Leeuwen, E.B., et al., *Expression of aberrantly glycosylated tumor mucin-1 on human DC after transduction with a fiber-modified adenoviral vector.* Cytotherapy, 2006. **8**(1): p. 24-35.

[2] Van Elssen, C.H., et al., *Expression of aberrantly glycosylated Mucin-1 in ovarian cancer.* Histopathology, 2010. **57**(4): p. 597-606.

[3] Van Elssen, C.H., et al., *Flow cytometry-based assay to evaluate human serum MUC1-Tn antibodies.* J Immunol Methods, 2011. **365**(1-2): p. 87-94.

Results continued

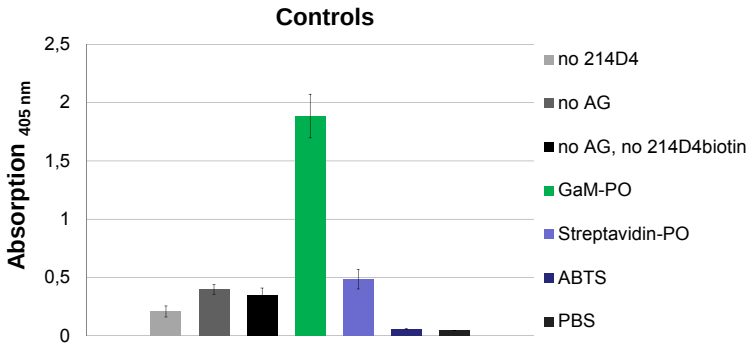

Fig.2: General detection of MUC1 on cells. Different negative controls were carried out, to exclude unspecific binding. Successful coating was shown by using a Goat α-Mouse antibody labeled with a horseradish peroxidase (GaM-PO, 1:5000). This analysis is representative of three independent experiments.

The set-up of the reference system was successfully performed by developing a cellular sandwich-ELISA with CHO-*ldl*D Muc1 cells as antigen (AG). As there are several epitopes on the cell surface, mAb 214D4 was used for coating (1µg/well) and in a biotinylated form for the detection (21ng). The titration of cells showed a mean absorption when using approximately 1x10^3 cells/well.

Therefore in a second step the experiment was reproduced in a glycosylation specific sandwich-ELISA using the breast cancer specific 5E5 mAb. GalNAc treated CHO-*ldl*D Muc1 cells and biotinylated mAb 5E5 for detection were used (Fig.2). The ELISA shows a primary detection of the breast cancer associated MUC1-variant after cell incubation with GalNAc. In comparison to the untreated antigen ELISA, the absorption of the glycosylated antigen is much lower, because of the decreased number of glycosylated epitopes or binding sites on the cell surface for a 5E5 detection. However, it could been demonstrated, that the mAb 5E5 showed no unspecific binding to the untreated antigen (-GalNAc) within the sandwich-ELISA experiments.

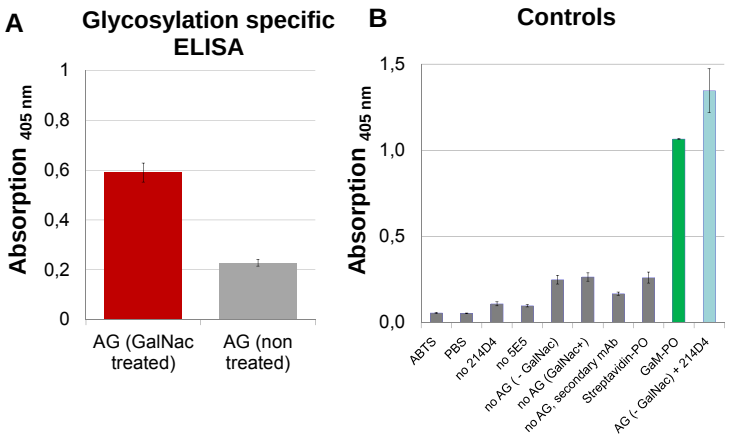

Fig.3: Cellular glycosylation specific analysis of MUC1 by ELISA.
The ELISA presents a detection of the underglycosylated MUC1 on cells using the specific 5E5 mAb. CHO-*ldl*D Muc1 cell line was treated with 1mM GalNac. The mAb 5E5 was used for coating (1µg/well) and the biotinylated form for detection (16 ng). To exclude unspecific binding, different negative controls were prepared. Two positive controls for the coating of 5E5 and the detection of the untreated antigen using 214D4 were carried out. This analysis is representative of three independent experiments.

Conclusions

- **Successful sandwich-ELISA with CHO-*ldl*D Muc1 cells as antigen using the mAb 214D4**
- **Detection of the cellular breast-cancer specific MUC1-variant using CHO-*ldl*D Muc1 cell line and mAb 5E5**

Project partners:

Funding:

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