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Published in:
Cancer Genomics & Proteomics

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Larsen, S. A., Meldgaard, T., Fridriksdottir, A. J. R., Lykkemark, S., Poulsen, P. C., Overgaard, L. F., ... Kristensen, P. (2016). Raising an Antibody Specific to Breast Cancer Subpopulations Using Phage Display on Tissue Sections. Cancer Genomics & Proteomics, 13(1), 21-30.

Download date: 17. Feb. 2019

Raising an Antibody Specific to Breast Cancer Subpopulations Using Phage Display on Tissue Sections

SIMON ASBJØRN LARSEN[1], THERESA MELDGAARD[2], AGLA JAEL RUBNER FRIDRIKSDOTTIR[3], SIMON LYKKEMARK[4,5], PI CAMILLA POULSEN[2], LAURA FALKENSTEEN OVERGAARD[2], HELENE BUNDGAARD PETERSEN[2], OLE WILLIAM PETERSEN[3] and PETER KRISTENSEN[2]

[1]*Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark;*
[2]*Department of Engineering, Aarhus University, Aarhus, Denmark;*
[3]*Department of Cellular and Molecular Medicine, Centre for Biological Disease Analysis and Danish Stem Cell Centre, University of Copenhagen, Copenhagen, Denmark;*
[4]*Department of Clinical Medicine, Aarhus University, Aarhus, Denmark;*
[5]*Sino-Danish Centre for Education and Research (SDC), Aarhus, Denmark*

Abstract. *Background/Aim: Primary tumors display a great level of intra-tumor heterogeneity in breast cancer. The current lack of prognostic and predictive biomarkers limits accurate stratification and the ability to predict response to therapy. The aim of the present study was to select recombinant antibody fragments specific against breast cancer subpopulations, aiding the discovery of novel biomarkers. Materials and Methods: Recombinant antibody fragments were selected by phage display. A novel shadowstick technology enabled the direct selection using tissue sections of antibody fragments specific against small subpopulations of breast cancer cells. Selections were performed against a subpopulation of breast cancer cells expressing CD271$^+$, as these previously have been indicated to be potential breast cancer stem cells. The selected antibody fragments were screened by phage ELISA on both breast cancer and myoepithelial cells. The antibody fragments were validated and evaluated by immunohistochemistry experiments. Results: Our study revealed an antibody fragment, LH8, specific for breast cancer cells. Immunohistochemistry results indicate that this particular antibody fragment binds an antigen that exhibits differential expression in different breast cancer subpopulations. Conclusion: Further studies characterizing this antibody fragment, the subpopulation it binds and the cognate antigen may unearth novel biomarkers of clinical relevance.*

Correspondence to: Peter Kristensen, Department of Engineering, Aarhus University, Gustav WiedsVej 10, 8000 Aarhus C, Denmark. Tel: +45 87155468, e-mail: pk@eng.au.dk

Key Words: Phage display, biomarkers, breast cancer, tissue, CD271+, shadow stick.

Today it is widely recognized that breast cancer is a disease that exhibits a large degree of heterogeneity, between and within patients. The heterogeneity between patients is characterized as belonging to one of several histological subtypes. Within any given patient, heterogeneity exists between the primary lesion and metastasis. Even within individual primary tumors different subpopulations of cancer cells co-exist, each displaying diverse sets of properties. This well-documented intra-tumor heterogeneity greatly affects morphology, growth, propensity to form metastasis, therapeutic resistance and recurrence of the individual tumor (27). Genetic and epigenetic alterations, stochastic gene expression and fluctuations in signaling pathways are all contributing factors to the diversity between different cancer cells (22, 23, 28). The intra-tumor heterogeneity is also shaped by extrinsic factors inherent to the microenvironment in which the tumor resides (25). It has been shown, that progression of the tumor is influenced by the surrounding stroma cells, extracellular matrix, paracrine factors or local conditions such as hypoxia (8, 16, 35). The complex network of cellular interactions with the microenvironment plays an important role in the antigens expressed by the cancer cells and may also influence therapeutic response (13, 26).

Most often, the molecular sub-classification of breast cancer is based on observation of the expression of a limited number of prognostic and predictive markers. Among these the estrogen (ER) and progesterone (PR) hormone receptors and ERBB2 (HER2) are the ones most commonly used (5). With the introduction of modern sequencing and DNA-based technologies, it has been verified that multiple biopsies obtained from the same tumor may possess genomic variations (2). Even when considering the established markers, such as HER2 status, variance has been established within primary tumors (6, 10). This poses a real clinical

challenge. A definitive diagnosis can, therefore, be obstructed by intra-tumor heterogeneity. Furthermore, the heterogeneity provides undefined subpopulations of breast cancer cells, often with unknown response to therapy.

By expanding the number of clinical-relevant biomarkers, characterization of individual breast cancer subpopulations will be improved. Furthermore, it will enhance the ability to accurately stratify breast cancer patients, detect recurring disease and predict resistance to therapy.

Studies performed on primary tumors, recurrent/metastatic lesions, circulating tumor cells or breast cancer cultures all contribute to our understanding of breast cancer as well as aid in the identification of the "driver" aberrations responsible for tumor progression and metastasis. The search for biomarkers has taken numerous approaches involving genomic, transcriptomic, proteomic or metabolic studies (1, 3, 7, 9, 17, 18, 30, 31). In the present study, we propose to unearth novel biomarkers through isolation of breast cancer cell-specific antibody fragments by performing *in vitro* selection of antibody libraries directly on tissue sections. Display technologies such as phage display, is a widely used method to generate cell-specific recombinant antibody fragments. It allows for *in vitro* selection of recombinant antibody fragments against unknown target antigens represented in any kind of material, such as cells, tissue or blood (20, 32, 34). The phage display technology may generate antibody fragments which cannot be obtained through *in vivo* immunization or infection, as the isolation does not rely on immunogenicity of the antigens, but purely binding between antibody and antigen (4). Furthermore, this technology may be capable of detecting differentially expressed biomarkers (12, 14). Antibody fragments have proven to be valid alternatives to full-size IgG antibodies, and their discovery, including the identification of their corresponding antigens, is highly useful for development of new diagnostic tools and individualized treatment (11). Within the pharmaceutical industry, increased attention is being paid on recombinant antibody technologies (24). During the past four years 54 biopharmaceutical products have been approved, out of which 17 were monoclonal antibodies (37).

Herein, we isolated one antibody fragment, LH8, which in IHC experiments specifically binds breast cancer cell subpopulations. We applied a recently established method, which allows selections to be targeted against small subpopulations of primary breast cancer cells in tissue (20). We targeted a particular subpopulation of cancer cells, capable of both initiating tumors and forming a differentiation hierarchy, as observed in primary xenografts after transplantation in NOD SCID gamma mice (15). This subpopulation of potential breast cancer stem cells is characterized by the expression of CD271, a marker not exclusive to cancer cells. The discovery of novel antibody fragments specifically recognizing biomarkers defining breast cancer subpopulations has great clinical potential.

Materials and Methods

Tissue sections. Cryostat sections (6-8 μm) from snap-frozen biopsies of breast cancer patients and healthy donors were prepared as previously described (29). The use of human material has been reviewed by the Regional Scientific Ethical Committees for Copenhagen and Frederiksberg and approved with reference to (KF) (11) 263995 and for Region Hovedstaden H-2–2011-052 and H-2–2010-051. Tissue sections used for selections were fixed for 10 min in 3.7% formaldehyde (Sigma-Aldrich Denmark A/S Copenhagen, Denmark), washed in PBS and incubated twice for 7 mins in 0.01% Triton X-100 (Sigma-Aldrich Denmark A/S Copenhagen, Denmark). Nuclei were counterstained with hematoxylin (Sigma-Aldrich). Selections were performed on basal-like breast cancer tissue from patient 757 with the marker profile: (ER/PR–/–, cytokeratin (CK)17+, CK5+, low ErbB2, MM+ and CD271+). Tissue sections used for immunohistochemistry (IHC) were fixed in ice-cold methanol (Sigma-Aldrich) at –20˚C for 5 min. Tissue sections from four breast cancer patients were used, two basal-like breast cancers: P757 and P918 (ER^-, $CK17^+$, $CK5^+$, MM^- and $CD271^+$) and two luminal breast cancers: P761 (ER/PR $^{+/+}$, $CK17^-$, $CK5^+$, low ErbB2, MM^+ and $CD271^+$) and P686 (ER/$PR^{+/+}$, $CK17^-$, $CK5^-$, low ErbB2, MM^+ and $CD271^+$).

Target area identification by immunoperoxidase staining with anti-CD271. Briefly, multiple sections were cut. The middle section was methanol fixed and used for immunoperoxidase, while the other sections were formalin fixed, as described. The tissue from the middle section was encircled with a PAP pen liquid blocker and blocked for 5 min in Ultra V Block (TA-060-UB, Thermo Scientific, Roskilde Denmark). The tissue was incubated for 1 h with 50 μL mouse anti-p75 NGF Receptor antibody (anti-CD271) [ME20.4] 1:50 (Abcam, Cambridge, UK, #ab8877), washed three times with PBS- (Ca^{2+} and Mg^{2+} free). Then incubated with 50 μL Ultravision ONE HRP Polymer (Thermo Scientific) for 30 min, washed three times with PBS and finally incubated with 1 mg/mL 3´, 3´-diaminobenzidine tetrahydrochloride (Dako Denmark A/S, Glostrup, Denmark) in PBS with freshly added 1 μl/ml of 30% H_2O_2 (Sigma-Aldrich) for 10 min. The tissue slide was washed with PBS and distilled water before the nuclei were counter-stained with hematoxylin (Sigma-Aldrich). $CD271^+$ cancer cells were found within a cancer nest in the methanol fixed section. Corresponding areas were identified on the formalin-sections and used for shadow stick selection.

Shadow stick. The shadow sticks were fabricated from a pulled injection microcapillaries (Tritech Research, Inc., Los Angeles, USA). The metal discs were created by compressing sinter metal powder, kindly provided by Dansk Sintermetal (Haderslev, Denmark). The flat pieces of powdered metal were placed on a microscope slide and attached to the tip of a pulled glass capillary by drawing up a small volume of epoxy glue into the capillary. Subsequently, the glue was dispensed on top of a piece of metal with a desired size. This procedure was done with the capillary attached to the micromanipulator, to ensure the disc being glued to the stick in the correct angle. This allows positioning of the disc on top of the target area using micromanipulation equipment from

Narishige (Model MM-188, Nikon Denmark, København S, Denmark). A shadow stick with a diameter of approximately 150 μm was used in this study.

Shadow stick-selection of antibody fragments using phage display. The breast cancer tissue sections from P757were formalin-fixed, as described, and blocked for 1 h in 4% Marvel dried skimmed milk powder (MPBS) (19). The tissue slide was incubated with the phage library in a slide container containing 20 ml 2% MPBS overnight with gentle agitation. The single domain library "predator" was used which has diversity of 6.2×10^7 different antibody fragments and a titer of 10^{13} pfu/ml with a display level estimated to be 6.4% (21). Fifty μL phage stock were used per tissue section and incubated overnight. The slide was washed 10 min in PBS and two times 10 min in PBS with 10% glycerol (PBSG) with gentle agitation. The slide was dried, except from the target area, which was kept moist with approximately 10 μL PBSG. Using bright-field microscopy, the shadow stick was positioned above the target area. The slide was exposed to UV-C light (254 nm) for 5 min using a UV-C source (model UVSL-14P from UVP, Upland, CA, USA) positioned on a stand approximately 4 cm above the slide. Phage particles bound to the target area was eluted with 15 μL trypsin (1 mg/mL) for 15 min. Trypsin was aspirated and transferred to a tube before the area was washed 15 times with 50 μL PBSG, which was transferred to the eluate as well. For trypsin inactivation 50 μL fetal bovine serum were added to the eluate before storage at –20˚C.

Cell cultures. Myoepithelial cells were isolated from trypsinized organoids derived from normal breast tissue as described previously (36). Trypsinized cells were incubated with conjugated monoclonal antibodies NGFR (neurotrophin receptor, p75)/CD271-APC (ME20.4, 1:50, Cedarlane Laboratories, TriChem Aps, Skanderborg, Denmark) for 45 min at 4˚C. Then washed 2x in HEPES buffer supplemented with 0.5% BSA (bovine fraction V; Sigma-Aldrich) and 2 mM EDTA (Merck) and finally incubated with 1 μg/ml propidium iodide (Life Technologies) to separate live from dead cells before analysis and sorting, using a FACSAria I flow cytometer (BD Biosciences). Sorted cells were set-up in culture on collagen-coated flasks with BBMYAB medium (publication to follow). Establishment and culture of the $CD271^+$ subclone from the BT474 cancer cell line have previously been described (15).

Phage ELISA for screening and titration assay. Phage infection of E. coli and production of phage antibodies was performed as described previously (20). Initial screening of potential interesting phage antibodies was performed on $CD271^+$ cancer cells. Titration assay was performed simultaneously on both $CD271^+$ cancer cells and normal $CD271^+$ myoepithelial cells, which enabled the possibility to reject the phage antibodies binding to common antigens. As a positive control for the phage ELISA procedure the phage antibody 52 was used (12). As a negative control, a phage antibody specific against foetal epsilon-haemoglobin was included (33). For titration assay, phage antibodies of interest were produced in 50 ml TG-1 cultures and tested along the above mentioned controls in a series of six 4-fold serial dilutions, ranging from 10^{11} phages/well to 9.8×10^7, as described (20). Phage particles were quantified by measuring absorbance at 269 nm and 320 nm (21).

Expression and purification of soluble antibody fragments. To express the individual clones as soluble antibody fragments, clones were sub-cloned from the predator phage into a modified pET22b vector including c-Myc- and His-tag using *NcoI* and *NotI* restriction enzymes (Thermo Scientific) and T4 DNA ligase (Fermentas) before transformation into *BL21 Gold* (Agilent Technologies, Glostrup, Denmark). Expression was initiated with a 4-ml overnight culture in TB medium containing ampicillin (100 μg/mL) and glucose (4% w/v). The cultures were diluted 1:100 in 250 ml cultures and grown until OD600 of 0.6-0.8, then spun for 10 min at 4˚C and 4,000 rpm. The pellet was re-suspended into TB medium containing ampicillin (100 μg/ml) and IPTG (100 μg/ml) for induction, and grown 16-18 h at 30˚C and 200 rpm. The cultures was spun for 1 hour at 5,000 × *g* at 4˚C and the antibody fragments in the supernatant was precipitated with 30% w/v ammonium sulphate by incubation on a roller table at 4˚C overnight. The flasks were spun for 30 min at 5,000 × *g* at 4˚C and the pellet re-suspended in 40 ml TBS (pH 8) with approximately 400 U DNase I (Roche) including 5 mM Mg^+. The solution was sterile-filtered with 0.20-μm filters (GF pre-filters) and purified on HiTrap Protein A HP columns (GE Healthcare A/S, Brøndby, Denmmark). The fractions containing the antibody fragments were determined by SDS-PAGE, pooled into a 3.5-kDa MW dialysis tube (Spectrum Laboratories) and dialysed in 3 l TBS pH 7.5 at 4˚C overnight with gentle agitation. The dialyzed protein was transferred to 3-kDa MW VivaSpin columns (GE Healthcare) and spun down to a concentration of about 1 mg/ml measured on a NanoDrop spectrophotometer (Thermo Scientific). Purity was verified by SDS-PAGE and western blotting against c-Myc.

Immunohistochemical staining with soluble domain antibody fragments. The tissue sections were prepared as described earlier. The tissue was encircled with a PAP pen and blocked for 1 h with Ultra V Block (TA-060-UB, Thermo Scientific). Approximately 25 μg of antibody fragments were dissolved in Ultra V Block, 10% goat serum and 1:100 anti-CK19 to a total volume of 100 μl, and added to the encircled area. Incubation was performed for 3 h in humid chambers. The liquid was removed by aspiration and the slide washed four times for 1 min in PBS. The slide was incubated for 30 min in the dark with mouse Cy3 conjugated anti-c-Myc antibody [9E10] 1:250 (Sigma-Aldrich), Alexa Fluor 488 conjugated Goat anti-mouse IgG2a 1:500 (Invitrogen, ThermoScientific), DAPI 1:1,000 (Invitrogen) and 10% goat serum dissolved in Ultra V Block to 100 μL. Alternatively, the antibody fragments above, was replaced with an anti-Ki67 antibody (Abcam) in a dilution of 1:100 and as secondary antibody a goat anti-rabbit antibody coupled to Alexa 546 (Thermo Fisher) was added in dilution of 1:500. The slide was washed three times for 1 min in PBS and mounted with Flouromount mounting media (Sigma-Aldrich) and cover glass.

Results

Outline. The shadow stick selection procedure on cryostat tissue sections is based on our previous published work (20). An outline of the selection and screening approach applied in this study can be seen in Figure 1. The selections were performed with a novel single-domain phage antibody library termed "predator"(21).

Identifying the target area. Multiple selections were performed on sections from a single biopsy (P757). This biopsy had small well-defined tumor cell nests and rare

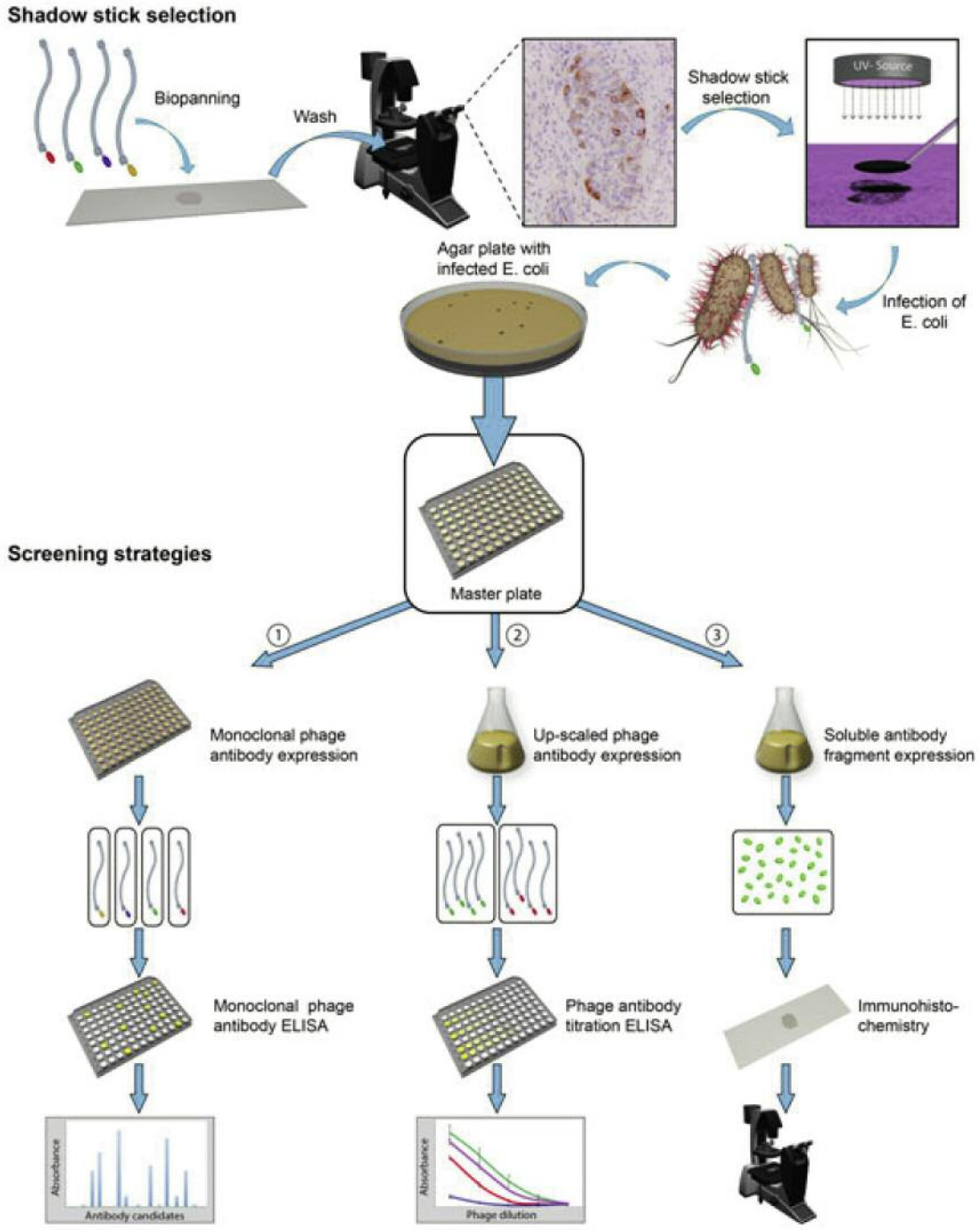

Figure 1. *Illustration of the antibody fragment selection procedure and screening strategies. The target area for selection was chosen to be the middle part of a particular cancer nest due to clustered CD271⁺ staining present. The entire tissue on a consecutive formalin-fixed section was then incubated with a phage library. The target area was relocated and a minute disc (shadow stick) was positioned precisely above the target cells of interest. The target area was kept moist at all times. The shadow stick shielded the phage antibodies binding to cells of interest from UV-C irradiation. The phages were eluted, but only those protected by the shadow stick can replicate in bacteria and provide ampicillin resistance. Each colony represented an antibody fragment, which required screening for their specificity. They were picked and grown in separate wells of a master plate. 1) In the initial screening, all colonies were grown in microtiter plates and monoclonal phage antibodies produced. The phage antibodies were tested by phage ELISA on CD271+ cancer cells. 2) All phage antibodies bound with higher affinities than the negative control in the initial screening were produced monoclonally in 50-ml cultures. These were tested in different concentrations by a phage ELISA titration assay, which was performed simultaneously on CD271⁺ cancer cells and CD271+myoepithelial cells. This provided comparative results of each phage antibody. 3) Soluble antibody fragments were expressed and purified, and examined by IHC experiments on four different breast cancer biopsies to validate their specificity.*

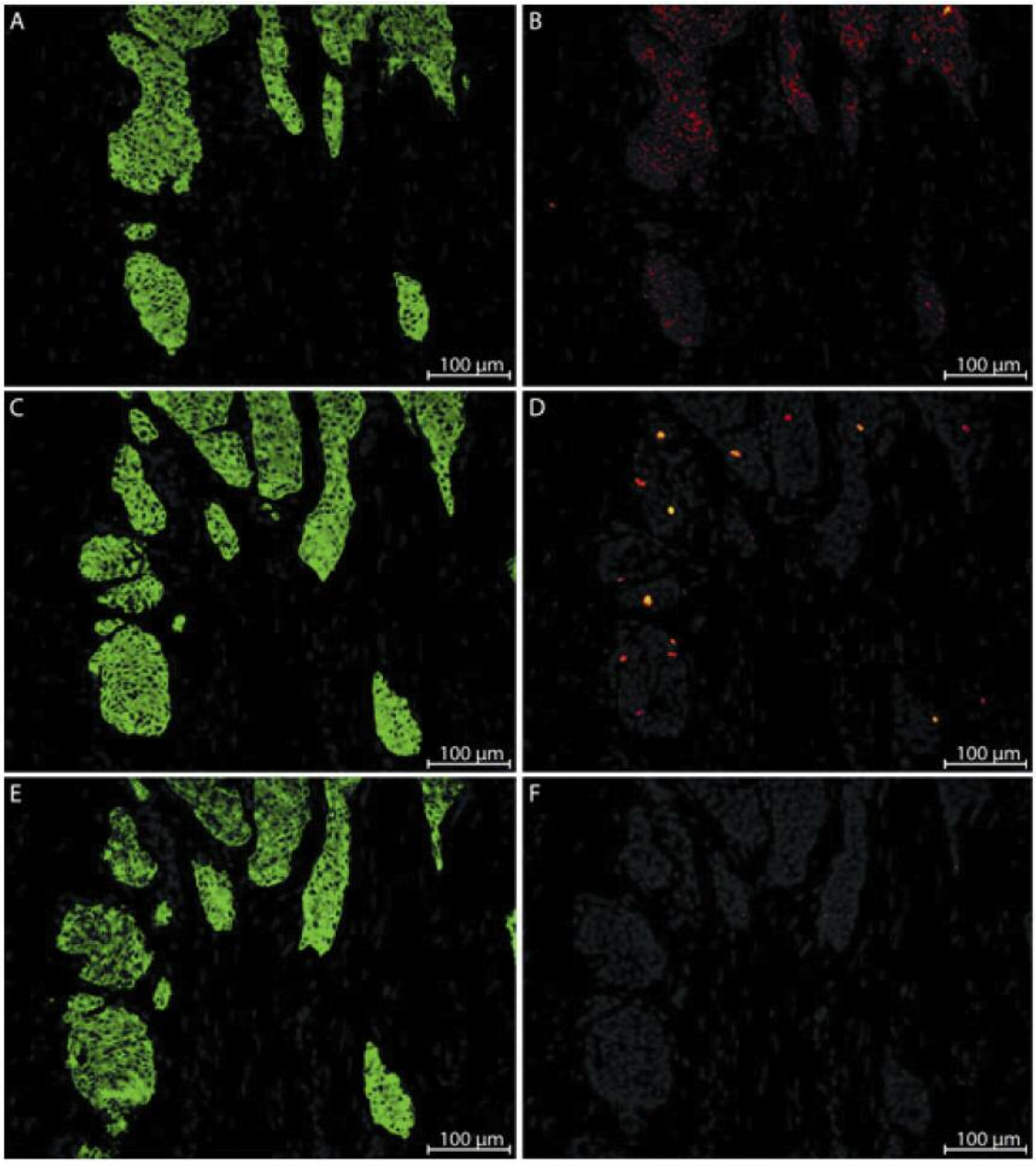

Figure 2. *Immunohistochemistry showing breast cancer-specific staining with an antibody against fragment LH8. Immunohistochemistry was performed on three seperate sections of cryostat tissue from the luminal breast cancer patient 761. All pictures are merged with DAPI staining. Pictures A, C and E show staining against CK19, which indicate the presence of cancer cells. Picture B shows staining with the antibody fragment LH8 which consistently only binds cancer cells. The presence of cancer cells is confirmed by staining with the proliferation marker ki67 within these areas, as observed in picture D. Picture F shows staining with the mouse Cy3 conjugated anti-c-Myc antibody used for detection of the antibody fragments. This shows that the observed staining is not caused by up-regulated c-myc expression in cancer cells or unspecific binding by this secondary antibody.*

$CD271^+$ staining throughout the tissue. The target area was chosen to be a high-density cluster of $CD271^+$ cells within a single cancer nest (Figure 1). The shadow stick shielded roughly 75-100 cells. The antigens that are presented in target cells might experience structural changes in the multiple steps of the CD271 immunostaining. To prevent this, the selection was performed on separate, but consecutive non-stained tissue sections. The consecutive slides used for selection were formalin-fixed and used fresh. During all steps of the selection procedure, the target area

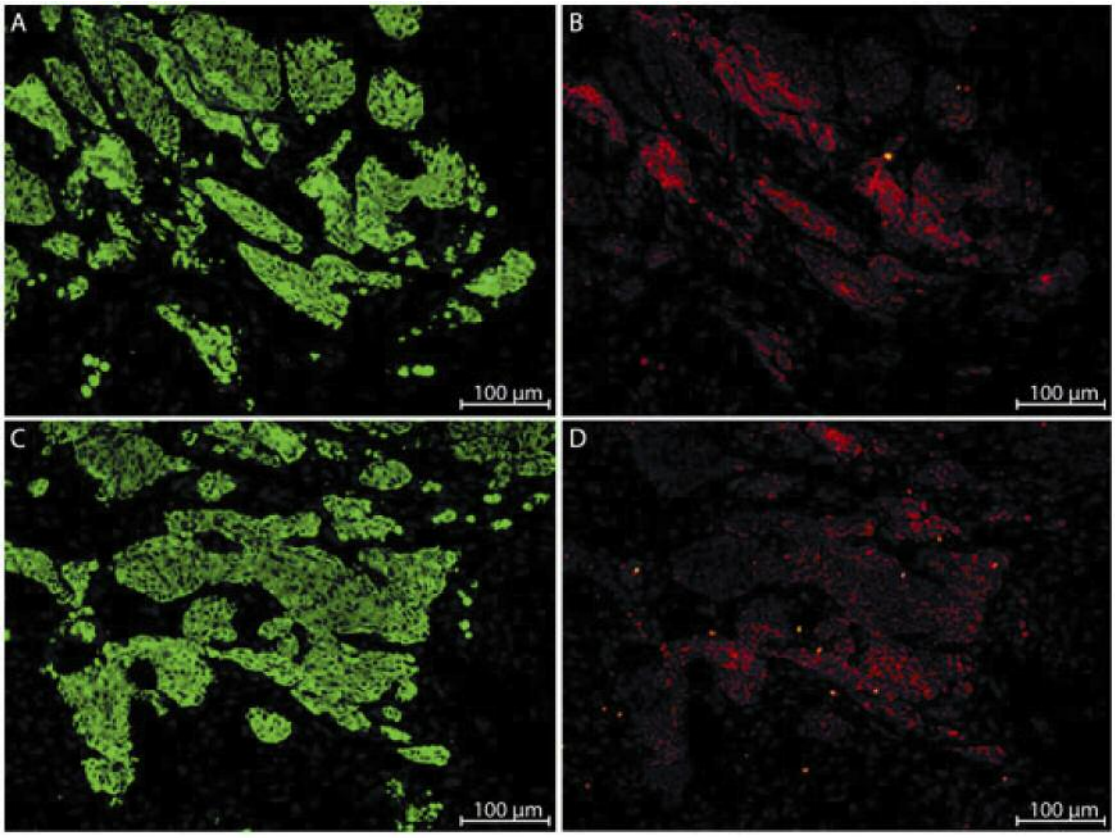

Figure 3. *Immunohistochemistry showing differential staining intensity in patient 761. The figure shows two different areas on the same section of a cryostat tissue from the luminal breast cancer patient 761. All pictures are merged with DAPI staining. Pictures A and C show staining against CK19. Picture B and D shows staining with the antibody fragment LH8. Although the pictures are from the same tissue section there exist variations in the staining pattern. Cancer cells are not uniformly stained but rather display different areas with different intensity. Some areas of cancer cells present very limited staining (Figure D, upper left and right) whereas others are very intense.*

was kept moist. The exact corresponding target area was easily identified on the neighboring slides, by the unique morphology patterns of the tumor cell nests.

Selection and screening. As the number of phage antibodies retrieved in a selection varies, multiple selections were performed in parallel. In the present study13 selections were performed on cryostat sections from the breast cancer patient 757. In total, 315 clones were screened by phage ELISA on CD271-expressing cancer cells with phage antibodies produced in a 96-well format. The phage antibody "Epsilon" is specific against epsilon-haemoglobin almost exclusively expressed by fetal erythroblasts (33). This phage antibody was included as a negative control in the initial ELISA screening. Phage antibodies which did not give a signal above the signal obtained from the negative control Epsilon was discarded from further analysis. The initial ELISA screen serves the sole purpose of prioritizing clones for further analysis. In total, 35 clones gave higher absorbance compared to the negative control Epsilon. The selection outputs of the predator library were generally of good quality and sequencing of the individual genes encoding these 35 antibodies of interest revealed no stop-codons, truncations, frameshift mutations or other abnormalities, as expected. The 35 phage antibodies were produced in 50-ml cultures and tested by titration phage ELISA in dilution series, as described. This was performed simultaneously on both $CD271^+$ cancer cells and $CD271^+$ myoepithelial cells. This allows for comparison between the individual phage antibodies and the two cell lines. The phage antibodies that bound equally well or better to myoepithelial cells were considered as common-epitope binders and not tested further. The phage antibody LH8, exhibited preferential binding to CD271+ cancer cells compared to the $CD271^+$ myoepithelial cells. Eleven out of the 35 tested phage antibodies were prioritized for further validation as they bound better to the

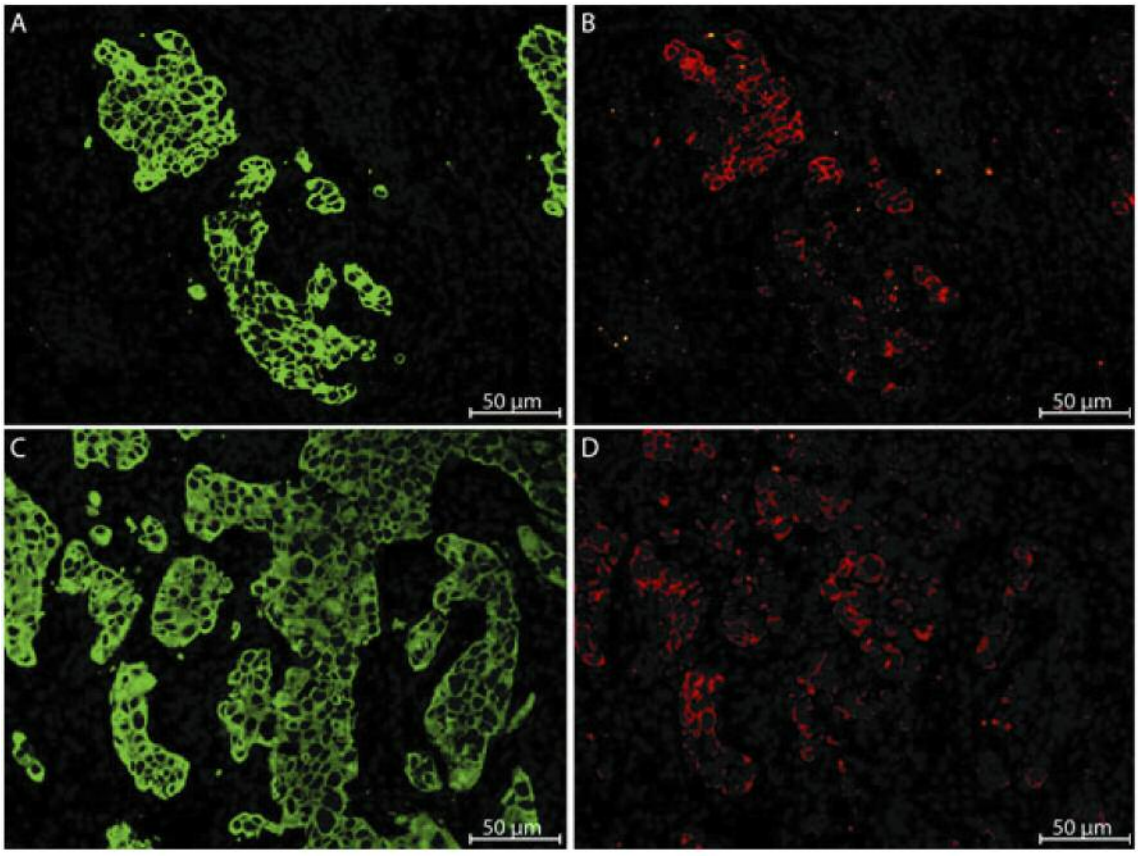

Figure 4. *Immunohistochemistry showing differential staining intensity in patient 686. The figure shows two different areas on cryostat tissue from luminal breast cancer of patient 686 at higher magnification. All pictures are merged with DAPI staining. Pictures A and C show staining against CK19. Pictures B and D show staining with LH8. The pictures are representative and display an obvious difference in staining intensity in different cancer areas.*

$CD271^+$ cancer cells compared to $CD271^+$ myoepithelial cells. Soluble antibody fragments were expressed and purified before validation by IHC.

Test of antibody specificity by immunohistochemistry and ELISA. IHC was performed with 11 soluble antibody fragments on cryo-preserved breast tissues from biopsies of four different cancer patients and three healthy donors. All tissue sections were co-stained with anti-CK19 to distinguish cancer cells with luminal characteristics from surrounding stroma. The majority of antibody fragments did not display cancer-specific staining, whereas the antibody fragment LH8 consistently showed staining restricted within tumor cell nests (Figure 2). This staining pattern indicated that this particular antibody fragment binds a cancer-associated antigen. Furthermore, LH8 stained a majority of cancer cells but with clear differences in intensity in different regions of tumor cell nests (Figures 3 and 4), staining was almost absent in certain cancer areas. This indicates that the antigen, to which LH8 binds, is expressed differently in different sub-populations of breast cancer. Apparently, the staining pattern of this antibody fragment does not distinguish between basal-like and luminal cancers. It bound cancer cells in all different cancer biopsies it was tested on (data not shown) and all displayed higher intensity in certain areas. IHC performed on breast tissue from three healthy donors did not show any binding with LH8 (Figure 5). As the staining pattern with LH8 coincided to some degree with CK19, this antibody fragment was also tested against coated full length human CK19 (ab73639) by ELISA. No reactions were observed in the ELISA experiments (data not shown), while this does not exclude binding of selected antibodies to CK19 due to other conformations of the epitopes in tissue, it is indicative of an alternative antigen being bound. Furthermore, IHC performed on healthy breast tissue showed no co-staining with any of the rare CK19-positive cells present. Taken together these results indicate that the antibody fragment LH8 binds to an alternate antigen.

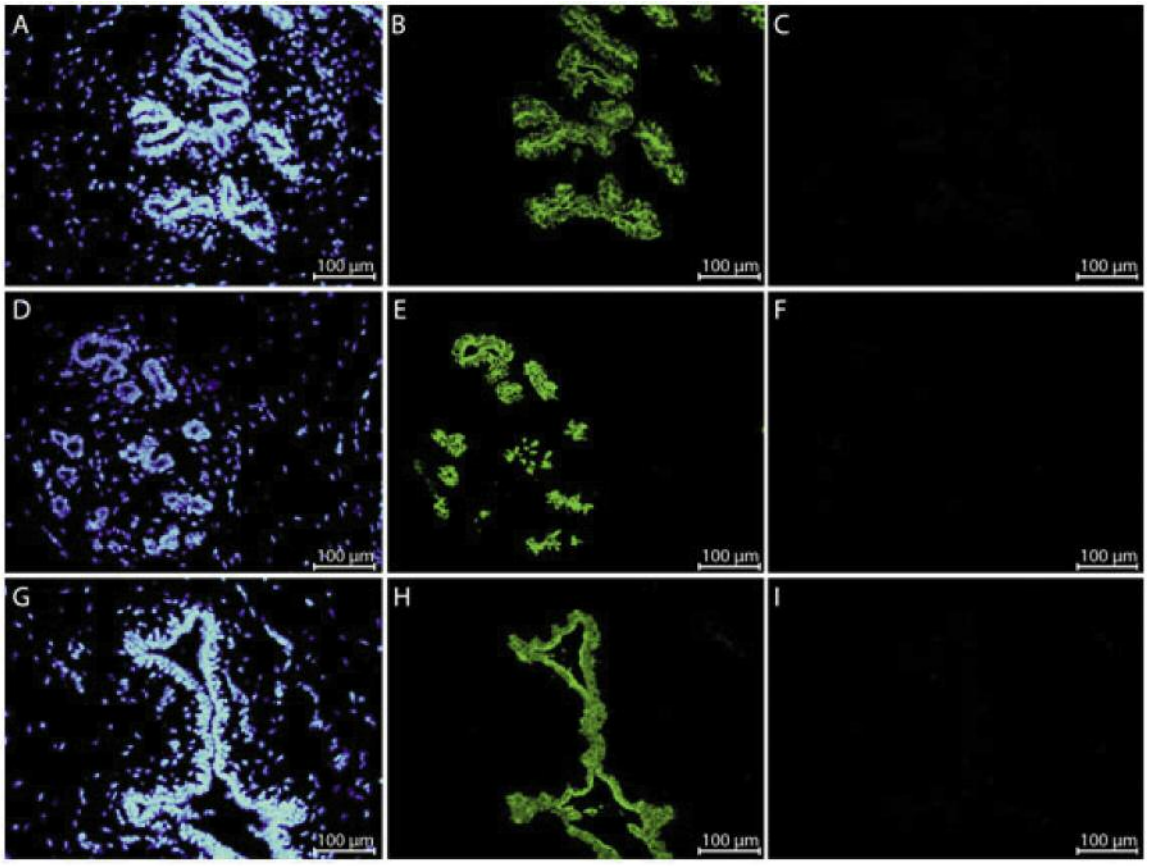

Figure 5. *Immunohistochemistry performed on healthy breast tissue. For further characterization of LH8, immunohistochemistry was performed on tissue sections from three healthy donors. Picture A, D and G show DAPI staining. B, E and H show staining against CK19. Picture C, F and I show staining with the antibody fragment LH8. The upper, middle and lower rows represent the healthy donors P671, P820 and P923 respectively. No staining was observed in neither the healthy luminal cells or stroma.*

Discussion

The isolation of cancer-specific antibody fragments is an initial step in the discovery of novel biomarkers for breast cancer. This article describes an antibody selection procedure where phage display of antibody fragments is applied on tissue. The advantage of such selections is that the tumor microenvironment is preserved and allows for selection of antibody fragment binding to antigens expressed at very specific locations within the tumor. The shadow stick shields phage particles binding to the target area from UV-C exposure, whereas all phage particle binding to areas outside the area protected by the shadow stick will experience cross-linking of their DNA and are, therefore, non-replicable in *E. coli*. Generally, this targeted-selection approach yields a low amount of output clones from each selection, but the frequency of phage antibodies binding to unique or up-regulated antigens is relatively high compared to traditional selections. This is due to the fact that only one round of selection is performed, thereby preserving diversity of binders and decreasing the bias toward selection of antibodies that bind with high affinity to highly expressed common antigens.

In the present study, multiple selections were performed in parallel on sections from a single biopsy. In this biopsy a cluster of $CD271^+$ cells within a single cancer nest were targeted for selection. Potentially, these $CD271^+$ cells can be classified as breast cancer stem cells (15). To preserve the structural presentation of antigens, the $CD271^+$ target area was identified on a separate slide, and the selections performed on consecutive neighbouring sections. As $CD271^+$ cells were targeted for selection on tissue, the screening of recombinant antibodies were likewise performed on $CD271^+$ breast cancer cells. As $CD271^+$ status is common in myoepithelial cells surrounding tumor cell nests in the tissue, these cells were convenient for the purpose of identifying and discarding common epitope binders, including binders to the antigen $CD271^+$. Titration assays were performed

simultaneously on both $CD271^+$ cancer cells and $CD271^+$ myoepithelial cells and it was the binding difference between the two cell types that was of interest.

The selected antibody fragment LH8 repeatedly displayed cancer-specific binding by IHC. Furthermore, this binding showed a great degree of variability in different areas of the tumor, indicating that the antigen being recognized by the antibody fragment is expressed differently by the various breast cancer subpopulations (Figures 2-4). No particular staining was observed in the surrounding stroma nor in breast tissue sections from healthy donors (Figure 5). The identification and validation of the cognate antigen is often a challenging and time-consuming task, but will provide significant knowledge to cancer biology. Typically, this would be performed by mass spectrometry analysis or screening of commercial protein micro-arrays or cDNA expression libraries. The chemical nature of the epitope to which the antibody fragment LH8 binds could range from being a linear polypeptide epitope to a conformational epitope or include post-translational modifications, such as glycosylation.

New biomarkers and therapeutic reagents are urgently required to improve breast cancer patient stratification and targeted therapy. While traditional genomics and proteomics strategies have provided and will continue to provide such biomarkers, we have taken an alternative approach relying on the selections of recombinant antibody fragments on tissue using phage display and shadow stick technology. This technology allows selection of recombinant antibody fragments specific for any kind of antigen presented by rare cells mixed within a heterogeneous population. The selected antibody fragment LH8 showed differential staining intensity in different areas within tumor cell nests and did not show staining of normal breast tissue. This indicates that the cognate antigen is differentially expressed or post-translationally modified in different subpopulations of breast cancer cells. Future characterization of these breast cancer subpopulations and identification of the cognate antigen may provide new insight of clinical relevance and aid in the development of new strategies for diagnosis and targeted therapy.

Conflicts of Interest

The Authors declare that they have no conflicts of interests.

Acknowledgements

The Authors thank Benedikte Thuesen, Københavns Privathospital and the donors for providing any normal breast biopsy material, and Vera Timmermans Wielenga, Pathology Department, Rigshospitalet for confirming the normalcy of the tissue and providing any cancer tissue.

The work was supported by Novo Nordisk Foundation (to DANSTEM), and Danish Research Council (to DANSTEM) (grant number 10-092798). The Danish Council for Research and Innovation (to PK) (grant number 09-069982) and the Danish Council for Independent Research Technology and Production Sciences to (PK) and the Sino-Danish center to (SL) (grant number 12–126975).

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Received September 5, 2015
Revised October 5, 2015
Accepted October 26, 2015