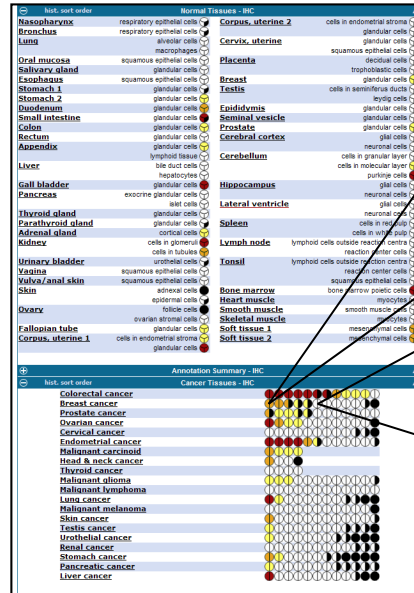


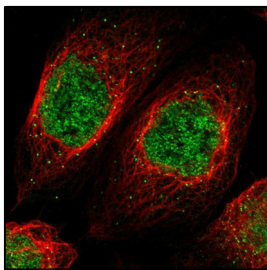
Biomarker discovery

The publicly available Human Protein Atlas (www.proteinatlas.org) gives the opportunity to use antibody-based proteomics to discover biomarkers of potential clinical relevance in a high-throughput manner. The advanced search tool of the Human Protein Atlas makes it feasible to find proteins with interesting expression patterns for extended studies and thereby the possibility to discover biomarkers of potential clinical utility (Björling, E. *et al. Mol cell Proteomics* 7(5), 825-844 (2008)). Proteins that show a specific expression pattern or a differential expression among the cancer samples in the Human Protein Atlas are selected to be further examined in extended tissue microarrays (TMAs). By this approach, we have identified several proteins with potential prognostic and/or response predictive implications in different types of cancer as well as cell type specific proteins.

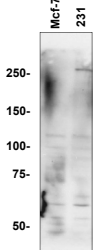
Within the HPR project, numerous extended TMAs containing different types of tumors have been made, but also TMAs containing several other disease areas such as ulcerative colitis, metabolic diseases and epilepsy. Approximately 5% of all HPA antibodies binds to proteins that show a specific or a differential immunohistochemical expression pattern in the Human Protein Atlas. These proteins are further investigated on a screening TMA, containing tissue from 60-200 patients. Each TMA consist of tissue from normal specimen, primary tumors of varying grade and subtype as well as lymph node and distant metastasis. The patient cohorts contain data on important clinicopathological parameters as well as information on survival and recurrence. Data on cell lines, western blot and immunofluorescence as well as existing literature are taken into consideration when evaluating the result. A protein that shows interesting data from the screening array is in general further examined by immunohistochemistry in a larger independent patient cohort, containing tissue from approximately 500 patients. Chi-square test and Pearson's correlation test are used for comparison of protein expression and patient- and tumor characteristics. Log rank test is used for Kaplan-Meier analysis and a Cox proportional hazard model is used for estimation of relative risks in univariate- and multivariate analysis. Calculations are performed with SPSS (SPSS Inc. USA).



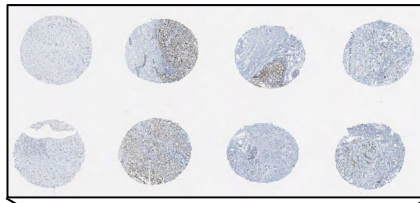
Immunohistochemical protein expression profile of HPA012672 in formalin fixed paraffin-embedded normal and cancer tissues. This protein showed a differential expression pattern in breast cancer.



Immunofluorescent staining of A-431 cell line showing nuclear positivity.

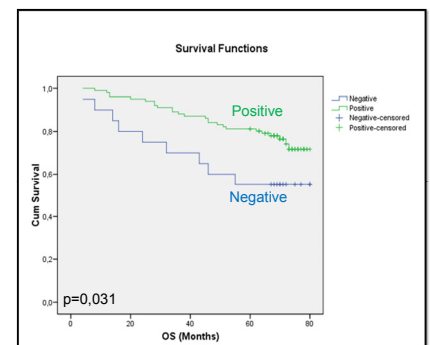


Western blot showing a weak specific band of 79 kDa, which is the estimated molecular weight for HPA012672, as well as other weak unspecific bands not corresponding to the predicted size.



Three TMAs showing the breast cancer screening cohort containing tissue from 144 breast cancer patients. A part of a sectioned TMA, stained with HPA012672 is seen on top.

Breast cancer screening cohort (N=144)



A positive nuclear expression of HPA012672 in breast cancer was significantly associated with a better outcome in overall survival (p=0,031).

