

**Systematic validation of novel breast cancer progression-associated biomarkers *via* high-throughput antibody generation and application of tissue microarray technology: An initial report.**

**Tumor Biology and Human Genetics**

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[\[Abstract\]](#)

**Background:** Omic-based discovery approaches, such as DNA microarray-based gene expression profiling, have provided powerful tools for biomarker identification. The limited availability of antibodies is a key barrier to the application of tissue microarrays (TMAs) for biomarker identification. To circumvent this issue, the Swedish Human Proteome Resource (SHPR; [www.proteinatlas.org](http://www.proteinatlas.org)) is using a high-throughput method to generate affinity-purified, mono-specific antibodies against all non-redundant human proteins.

**Methods:** We applied a novel bioinformatic technique to in-house, and publicly available DNA microarray datasets relating to breast cancer invasion and metastasis and identified a cohort of several hundred candidate progression-associated biomarkers. Of these genes, 137 targets were then selected for antibody production within the SHPR. In Feb. 2007, an initial 32 antibodies were released for extended analysis. Antibodies were screened *via* Western blot (WB) analysis and immunohistochemistry (IHC) against a range of normal (48) and tumor tissues (20), as well as cultured cells (66), represented on TMAs. Highly optimised antibodies were screened against a TMA constructed from a cohort of 512 consecutive breast cancer cases. The median age was 65 years and median follow-up time was 11 years. All invasive TNM stages were represented within the cohort.

**Results:** Approximately 25% of the initial antibodies show differential staining between normal and breast cancer tissue. PDZK1, an estrogen-responsive gene associated with good prognosis at the transcript level in breast tumors showed discrete banding at the predicted molecular weight (57 kDa) in estrogen receptor (ER)-positive breast tumor cell lines. The differential expression of PDZK1 protein between ER+ and ER- cell lines was also confirmed by IHC. Expression of PDZK1 protein was associated with improved breast cancer-specific survival ( $p=0.0247$ ), ER positivity ( $p=0.041$ ) and low grade ( $p=0.002$ ).

**Conclusions:** We have developed a comprehensive biomarker pathway that extends from discovery to validation on TMA and is yielding clinically relevant biomarkers.