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Claudin-2 is an independent negative prognostic factor in breast cancer and specifically predicts early liver recurrences

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ABSTRACT

Background: Predicting any future metastatic site of early-stage breast cancer is important as it significantly influences the prognosis of advanced disease. This study aimed at investigating the potential of claudin-2, over-expressed in breast cancer liver metastases, as a biomarker for predicting liver metastatic propensity in primary breast cancer.

Methods: Claudin-2 expression was analyzed in two independent cohorts. Cohort 1 included 304 women with metastatic breast cancer diagnosed between 2002 and 2007, while cohort 2 included 237 premenopausal women with early-stage node-negative breast cancer diagnosed between 1991 and 1994. Global transcriptional profiling of fine-needle aspirates from metastases was performed, followed by immunohistochemical analyses in archival primary tumor tissue. Associations between claudin-2 expression and relapse site were assessed by univariable and multivariable Cox regression models including conventional prognostic factors. Two-sided statistical tests were used.

Results: *CLDN2* was significantly up-regulated ($P < 0.001$) in liver metastases compared to other metastatic sites. Claudin-2 protein was more frequently expressed in primary tumors from patients who subsequently developed liver metastases ($P = 0.02$) and high expression was associated with a shorter metastasis-free interval (cohort 1, HR = 1.4, 95% CI = 1.0–1.9; cohort 2, HR = 2.2, 95% CI = 1.3–3.5). Specifically, a significantly shorter interval between primary tumor diagnosis and liver-specific recurrence was observed among patients with high levels of claudin-2 expression in the primary tumor (cohort 1, HR = 2.3, 95% CI = 1.3–3.9).

Conclusion: These results suggest a novel role for claudin-2 as a prognostic biomarker with the ability to predict not only the likelihood of a breast cancer recurrence, but more interestingly, the liver metastatic potential of the primary tumor.

Abbreviations: BCSS, breast cancer specific survival; CI, confidence interval; *CLDN2*, claudin-2 mRNA; ER, estrogen receptor; HR, hazard ratio; IHC, immunohistochemistry; LiMFS, liver metastasis-free survival; LNM, lymph node metastasis; OR, odds ratio; PR, progesterone receptor; RFS, relapse-free survival; TMA, tissue microarray.

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1. Introduction

Despite advances in management and the favorable prognosis of patients with early breast cancer, metastases are frequently diagnosed and the anatomical location of the metastases is correlated to the length of survival after recurrence (Imkampe et al., 2007; Largillier et al., 2008; Yardley, 2010). With the exception of the brain, recurrence in the liver is prognostic of the worst outcome relative to loco-regional, bone or lung relapses (Goldhirsch et al., 1988; Imkampe et al., 2007; Pentheroudakis et al., 2006; Yardley, 2010). Approximately 50% of all patients diagnosed with metastatic breast cancer develop hepatic metastases (Mano et al., 2005; Singletary et al., 2003; Solomayer et al., 2000) and there is evidence purporting an increasing trend in breast cancer liver metastases (Kennecke et al., 2010). However, the molecular determinants of site-specific metastatic preferences and factors accounting for heterogeneity in response to treatment and outcome are yet to be comprehensively established. A better understanding of these factors will likely influence decisions about surveillance and adjuvant therapy, as well as treatment of advanced disease.

Conventional clinico-pathological markers are used to assess the risk of recurrence. In addition, gene expression signatures stratifying patients according to recurrence risk (reviewed in Sotiriou and Pusztai, 2009) and more specifically, predicting the propensity of relapsing in bone (Kang et al., 2003), lung (Minn et al., 2005) and brain (Bos et al., 2009) have been published. However, because experimental models incompletely capture the relevant genetic complexity and the contribution of the host tumor microenvironment, studies using biopsies from metastases may be more suitable for identifying site-specific predictive biomarkers. Recently, we performed comparative genome-wide transcriptional profiling of a consecutive series of breast cancer metastases with one of the specific objectives being to identify potential liver metastasis genes (Kimbung et al., unpublished results). Remarkably, we observed that contrary to the down-regulation of many genes involved in cell adhesion and matrix re-modeling in liver metastases, *CLDN2*, a member of the same gene family, was significantly over-expressed. Over-expression of *CLDN2* was also recently observed in an experimental mouse model of breast cancer liver metastases (Tabaries et al., 2011), as well as in a limited series of clinical samples of breast cancer liver metastases (Tabaries et al., 2012), with accompanying data supporting the involvement of claudin 2 in the establishment and out-growth of breast cancer cells in the liver microenvironment. These data motivated the design of the present study, which was aimed at investigating if the high expression of *CLDN2* observed in liver metastases, is also a trait of primary breast cancers that recur in the liver. Furthermore, we sought to explore associations with conventional prognostic factors for breast cancer and patient outcome, with particular focus on the potential of claudin-2 as a biomarker for predicting liver metastatic propensity in primary breast cancer.

2. Materials and methods

2.1. Patients and tumors

This study was approved by the regional ethics committees at all participating sites.

2.1.1. Cohort 1

The test cohort consisted of 304 women with metastatic breast cancer who were enrolled in a randomized phase III trial conducted between 2002 and 2007 in Sweden, comparing two different first-line chemotherapy regimens (Hatschek et al., 2012). Patients with brain metastases, HER2 amplified tumors, or other malignancies diagnosed within five years of enrollment were excluded from the trial. Complete information on the study design, patient characteristics and trial outcome has been reported (Hatschek et al., 2012). The median follow-up for the endpoints relapse free survival (RFS) and breast cancer specific survival (BCSS) was 6.0 and 9.7 years respectively, for patients alive at last update.

2.1.2. Cohort 2

The prognostic value of claudin-2 was further evaluated in an independent cohort of 237 premenopausal women with early-stage lymph-node negative breast cancer included in a prospective study evaluating the prognostic value of the S-phase fraction (Malmstrom et al., 2001). Adjuvant treatment was administered to only 29 (12%) patients. Detailed information on treatment and evaluation of tumor pathological markers has been previously reported (Klintman et al., 2010; Malmstrom et al., 2001). Median follow-up was 10.6 and 18.3 years for RFS and BCSS, respectively.

2.2. Transcriptional analyses

Fine-needle aspirates from metastatic lesions from different anatomical sites were collected prior to treatment of metastatic disease whenever possible (cohort 1) and subjected to whole-genome transcriptional profiling. Tumor cellularity was assessed by a pathologist on Giemsa stained, ethanol-fixed, cytospin preparations and only samples with high (>50%) tumor cell content were included in the final analyses. Total RNA was extracted using the Qiagen RNA Mini kit (Qiagen, Valencia, CA), integrity analyzed using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA) and hybridized onto custom made Affymetrix HuRSTA-2a520709 gene chips. Raw intensity gene expression levels were processed and normalized using the robust multichip average (RMA) algorithm. After normalization, a probe presence filter was applied to select only probes present in ≥90% of assays. Probes expressed below the median intensity of Y-chromosome genes were filtered out of the dataset and gene-specific expression intensities were summarised by merging probes based on gene symbol. Finally, data were Log 2 transformed and mean-centered across the entire dataset. All data processing and normalization steps were performed in the R environment (www.r-project.org). Ninety-one out of 120 samples passed all quality assessments and were included in