PURPOSE: We have shown that DNA methylation of the PITX2 gene predicts risk of distant recurrence in steroid hormone receptor-positive, node-negative breast cancer. Here, we present results from a multicenter study investigating whether PITX2 and other candidate DNA methylation markers predict outcome in node-positive, estrogen receptor-positive, HER-2-negative breast cancer patients who received adjuvant anthracycline-based chemotherapy. EXPERIMENTAL DESIGN: Using a microarray platform, we analyzed DNA methylation in regulatory regions of PITX2 and 60 additional candidate genes in 241 breast cancer specimens. Using Cox regression analysis, we assessed the predictive power of the individual marker/marker panel candidates. Clinical endpoints were time to distant metastasis, disease-free survival, and overall survival. A nested bootstrap/cross-validation strategy was applied to identify and validate marker panels. RESULTS: DNA methylation of PITX2 and 14 other genes was correlated with clinical outcome. In multivariate models, each methylation marker added significant information to established clinical factors. A four-marker panel including
PITX2, BMP4, FGF4, and C20orf55 was identified that resulted in improvement of outcome prediction compared with PITX2 alone. CONCLUSIONS: This study provides further evidence for the PITX2 biomarker, which has now been successfully confirmed to predict outcome among different breast cancer patient populations. We further identify new DNA methylation biomarkers, three of which can be combined into a panel with PITX2 to increase the outcome prediction performance in our anthracycline-treated primary breast cancer population. Our results show that a well-defined panel of DNA methylation markers enables outcome prediction in lymph node-positive, HER-2-negative breast cancer patients treated with anthracycline-based chemotherapy.