INTRODUCTION

Raf-1 Kinase Inhibitor Protein (RKIP) is a physiological endogenous inhibitor of the Raf-MEK-ERK pathway (1). Alterations in this pathway are associated with tumor development and progression. Recent work has shown that RKIP is diminished in some metastatic cancers. We studied lymph node negative cancers and node-positive patients, with paired lymph node metastases and found a highly significant downregulation of RKIP in lymph node metastases (p<0.001), compared to matched primary tumors. No significant correlations were found between RKIP expression and established clinico-pathological prognostic indicators, suggesting that RKIP expression is an independent marker of breast cancer lymph node metastases.

METHOD

Paraffin-embedded tumors from 103 patients (52 node-negative and 51 node-positive patients, with 51 paired lymph node metastases) underwent immunohistochemical staining for RKIP using the streptavidin–biotin method and the chromogen 3, 3-diaminobenzidine. Paraffin-embedded sections of prostate cancer served as positive RKIP controls and omission of primary antibody served as negative controls. RKIP expression levels were scored using the visual grading system.

RESULTS

All of the primary breast tumors analyzed in this study demonstrated RKIP immunoreactivity (except for one node-negative tumor), and the distribution of RKIP was found to be mainly cytoplasmic. RKIP expression was predominantly moderate in node-positive tumors (31/51 cases, 60.8%) and was weakly positive in 20/51 (39.2%) cases. By contrast, in the matched lymph node metastases RKIP expression was considerably diminished and, in some cases, was entirely absent (9/51, 17.7%), and this decrease was found to be highly significant (p=0.000003; Fig 1). In total, 30/51 (58.8%) of lymph node cases were weakly positive and 12/51 cases (23.5%) were moderately positive for RKIP expression. Of the node-negative tumors, RKIP expression was moderate in 37/52 cases (71.2%), weakly positive in 14/52 cases (26.9%) and negative in 1/52 cases (1.9%). A trend was observed for more intense RKIP staining of node-negative breast tumors than for node-positive tumors, but this was not found to be statistically significant.

Fig. 1. A node-positive breast carcinoma (A) and the matched lymph node metastasis (C). Moderate RKIP immunoreactivity is present in the primary tumor (B), compared to very faint RKIP staining in the matched lymph node metastasis (D).
DISCUSSION
Recently, RKIP was identified as a candidate metastasis suppressor gene in prostate cancer and melanoma cell lines (2,3). Originally, RKIP was described as an inhibitor of the ERK pathway which is often hyperactivated in breast cancer (4). Therefore, we assessed RKIP in node-positive and node-negative breast cancers and found a highly significant diminution of RKIP expression in the metastatic lymph nodes, compared to the matched primary tumor. Taken together, our data demonstrate that RKIP expression is consistently downregulated in lymph node metastases of breast cancers. In addition, no significant correlations were found between RKIP expression and established clinico-pathological prognostic indicators, which suggests that RKIP expression is an independent marker of breast cancer lymph node metastasis. We therefore propose that re-activation of RKIP expression could provide a new chemotherapeutic strategy for the treatment of metastatic breast cancer.

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REFERENCES