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Akt2 expression is associated with good long-term prognosis in oestrogen receptor positive breast cancer

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Abstract ***Introduction:*** Akt is a signalling modulator for many cellular processes, including metabolism, cell proliferation, cell survival and cell growth. Three isoforms of Akt have been identified, but only a few studies have concerned the isoform-specific roles in the prognosis of breast cancer patients. The aim of this study was to investigate the prognostic value of v-akt murine thymoma viral oncogene homologue 1 (Akt1) and v-akt murine thymoma viral oncogene homologue 2 (Akt2) in oestrogen receptor positive (ER+) and oestrogen receptor negative (ER–) breast cancer with long-term follow-up.

Material and methods: The expression of Akt in tumour tissue was analysed with immunohistochemistry in a cohort of 272 postmenopausal patients with stage II breast cancer. The median follow-up time was 19 years. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using the Cox's proportional hazards model.

Results: The risk of distant recurrence was reduced for patients with ER+ tumours expressing Akt2 compared to patients with no Akt2 expression (HR = 0.49, 95% CI 0.29–0.82, $p = 0.007$). When adjusting for important clinical tumour characteristics and treatment, Akt2 was still an independent prognostic factor (HR = 0.38, 95% CI 0.21–0.68, $p = 0.001$)

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and the association remained long-term. The prognostic value of Akt2 increased with higher oestrogen receptor levels from no effect among patients with ER– tumours to 68% risk reduction for the group with high ER-levels (P for trend = 0.042). Akt1 showed no significant prognostic information.

Conclusion: Our results indicate that Akt2 expression is associated with a lower distant recurrence rate for patients with ER+ tumours and that this association remains long-term. The prognostic value of Akt2 increases with higher oestrogen receptor expression, motivating further mechanistic studies on the role of Akt2 in ER+ breast cancer.

1. Introduction

Akt is a signalling modulator for many cellular processes, including metabolism, cell proliferation, cell survival and cell growth.[1] Three isoforms of Akt have been identified: v-akt murine thymoma viral oncogene homologue 1 (Akt1), v-akt murine thymoma viral oncogene homologue 2 (Akt2) and v-akt murine thymoma viral oncogene homologue 3 (Akt3). Several studies have shown that Akt status influences the prognosis of breast cancer patients, but only a few are concerned with potential isoform-specific roles and there are conflicting results. A study on transgenic mice showed that Akt2 was a protective factor against tumour induction, whereas Akt1 had the opposite effect.[2] Another study in mice showed that Akt2 promoted metastases, whereas Akt1 impaired metastases.[3] This indicates not only that the different isoforms may have different functions, but also that the same isoform can take both protective and destructive roles possibly depending on the stage of breast cancer development. Studies on the potential different roles of the Akt isoforms have been reviewed.[4]

Phosphorylated Akt (pAkt) has been associated with up to 40% of breast cancers.[5] The mechanisms behind enhanced Akt phosphorylation are several, including human epidermal growth factor receptor 2 (HER2) amplification, phosphatidylinositol 3-kinase (PI3K) mutation and phosphatase and tensin homologue (PTEN) loss.[4] Most results suggest that pAkt correlates with poor prognosis[6,7] and is associated with other aggressive prognostic factors, such as HER2-positivity and lymph node positive breast cancer.[8] In a recent study, activated Akt1 was shown to drive progression in early breast cancers, whereas activated Akt2 may reverse this effect in cases where both Akt1 and Akt2 are activated.[9] Although pAkt expression is more closely related to Akt1 than to Akt2 in breast cancer,[8,10,11] it has been reported that Akt2 is also frequently upregulated in HER2-positive breast tumours and may contribute to tumour aggressiveness.[12] However, recent studies have shown that overexpressed Akt2 is a favourable prognostic factor.[11,13] In these studies, only patients with oestrogen receptor positive tumours were included.

In this paper we have investigated the prognostic value of Akt1 and Akt2 for stage II patients from a randomised study including both patients with oestrogen receptor-negative (ER–) and oestrogen receptor-positive (ER+) tumours, where only half of the patients with ER+ tumours received hormonal treatment. The aim was to compare the influence of the Akt isoforms in tumours with different hormonal status. We have also examined how the prognostic value of Akt1 and Akt2 changes over time.

2. Materials and methods

2.1. Patients

Between November 1976 and April 1990 both premenopausal and postmenopausal patients were randomised in a trial with the aim to compare postoperative radiotherapy with adjuvant chemotherapy. Only patients with tumour size $\geqslant 30$ mm and/or lymph node metastases were included. The postmenopausal patients were further randomised using a 2×2 factorial study design to one of four groups: adjuvant cyclophosphamide methotrexate fluorouracil (CMF) chemotherapy, adjuvant chemotherapy plus tamoxifen, postoperative radiotherapy or postoperative radiotherapy plus tamoxifen. The data material was previously described.[14] In this study we have used data from a subset of the postmenopausal patients consisting of 272 patients with median age 59 years (range: 45–71) for whom data on Akt1, Akt2 and pAkt were available from a previous study.[10] The period of follow-up has now been extended.

2.2. Immunohistochemistry

The expression of Akt1, Akt2 and pAkt was analysed by immunohistochemistry as previously reported.[10] Goat polyclonal antibodies against Akt1 and Akt2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, United States of America [USA]), and a sheep polyclonal antibody against the phosphorylated serine residue in position 473 of human Akt1 (Upstata Biotechnology, Lake Placid, NY, USA) were used for immunostaining.

The isoforms had different staining patterns. In the immunopositive tumours, Akt1 was frequently expressed in a high percentage of cells, whereas the staining of Akt2 was mostly sparse. Patients whose tumours showed staining of at least 1% of the cells were defined as Akt1+ and Akt2+, respectively.