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DNA-methylation of the homeodomain transcription factor PITX2 reliably predicts risk of distant disease recurrence in tamoxifen-treated, node-negative breast cancer patients – Technical and clinical validation in a multi-centre setting in collaboration with the European Organisation for Research and Treatment of Cancer (EORTC) PathoBiology group

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ABSTRACT

Our aim was to identify and validate DNA-methylation markers associated with very good outcome in node negative, hormone receptor positive breast cancer patients after adjuvant endocrine therapy which might allow identifying patients who could be spared the burden of adjuvant chemotherapy. Using a methylation microarray, we analysed 117 candidate genes in hormone receptor-positive tumours from 109 breast cancer patients treated by adjuvant tamoxifen. Results were validated in an independent cohort ($n = 236$, 5 centres). Independent methodological validation was achieved by a real-time polymerase chain reaction (PCR)-based technique. DNA methylation of PITX2 showed the strongest correlation with distant recurrence. Its impact on patient outcome was validated in the independent cohort: 86% of patients with low PITX2 methylation were metastasis-free after 10 years, compared to 69% with elevated PITX2 methylation. Moreover, PITX2 methylation added significant independent information to established clinical factors. All clinical and

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technical findings were confirmed by quantitative DNA-methylation PCR. These results provide strong evidence that DNA-methylation analysis allows clinically relevant risk assessment in tamoxifen-treated primary breast cancer. Based on PITX2 methylation, about half of hormone receptor-positive, node-negative breast cancer patients receiving adjuvant tamoxifen monotherapy can be considered low-risk regarding development of distant recurrences and may thus be spared adjuvant chemotherapy. In addition, these low-risk postmenopausal patients seem to respond sufficiently well to tamoxifen so that they may not require up-front aromatase inhibitor therapy.

1. Introduction

Current guidelines recommend adjuvant chemotherapy followed by endocrine therapy for most women with node-negative, steroid hormone receptor-positive breast cancer.[1] This recommendation is based on a significant reduction of the risk of disease recurrence by chemotherapy in this population, independent of the risk reduction by endocrine therapy.[2] However, these patients have a rather good prognosis and generally derive significant benefit from endocrine treatment alone.[2] Hence, after endocrine treatment, the majority will never suffer recurrence and thus would have been adequately treated by tamoxifen alone. Unfortunately, traditional prognostic factors are not adequate to identify those patients at low risk who can be spared over-treatment by chemotherapy, as is true in the majority of hormone receptor-positive, node-negative patients. In postmenopausal hormone receptor-positive patients, aromatase inhibitors have become an important treatment option.[3] Yet, it is still unclear which patients will be sufficiently treated by adjuvant tamoxifen and which will benefit more from aromatase inhibitors – a rather important question given the lack of information on long-term side effects and the increased costs for aromatase inhibitors.

A common and early event in cancer is aberrant DNA methylation of cytosine phosphoguanine dinucleotides (CpG) within gene regulatory regions.[4,5] Frequently, hypermethylation of promoters is associated with suppression of gene expression.[5,6] DNA-methylation patterns are tumour-specific and can be used for molecular subclassification of tumours.[7–9] Additionally, several studies have demonstrated the potential of DNA-methylation as prognostic or predictive markers in a variety of cancers.[10–12] Recently, some studies have suggested that methylation of certain genes may correlate with tamoxifen response and survival in breast cancer.[13,14]

Our aim was to identify and validate, for the first time, DNA-methylation markers associated with a low risk of distant recurrence in breast cancer patients receiving adjuvant tamoxifen monotherapy. Using a previously described microarray approach[7,14], we analysed DNA methylation of 117 candidate genes in primary tumours of 109 hormone receptor-positive breast cancer patients, all of whom had received tamoxifen as their sole adjuvant systemic treatment. The candidate genes were selected because of their potential function in breast tumourigenesis or metastasis, because of their presumed role in resistance to endocrine therapy or steroid hormone regulation, or because they had been described as being methylated in cancer. In the subsequent validation study, 33 candidates were selected and analysed in an independent cohort encompassing 236 node-negative, hormone receptor-positive patients from five clinical centres.

2. Materials and methods

2.1. Patient and tumour characteristics

DNA-methylation measurements were performed on DNA isolated from snap-frozen primary breast cancers. The unicentre marker discovery study included 39 node-negative and 70 node-positive breast cancer patients (Department of Obstetrics and Gynecology, Technical University of Munich, Germany) who underwent surgery in 1998 or earlier. In the multi-centre validation study, 236 independent node-negative breast cancer patients were analysed (Departments of Obstetrics and Gynecology, Technical University of Munich and University Hospital Eppendorf, Hamburg, Germany; Stiftung Tumorbank Basel, Switzerland; Clinical Experimental Oncology Laboratory, National Cancer Institute, Bari, Italy; and Laboratoire d'Oncogénétique, Centre René Huguenin, St. Cloud, France). Inclusion criteria were availability of frozen tumour specimens, T1–3, oestrogen receptor (ER) and/or progesterone receptor (PR) expression, no lymph node involvement (validation phase only), age > 35 years at diagnosis, surgery by 1998, adjuvant tamoxifen monotherapy (indicated duration 5 years), availability of follow-up data and written informed patient consent. Clinical patient characteristics are summarised in Table 1. Note that due to the lack of material (in most cases, only cellular nuclei available), tumour grade and oestrogen receptor protein levels could not be confirmed centrally, but data from medical records of each individual centre were used. Furthermore, tumour cell content could not be determined. However, since the cellular nuclei originated from specimens used for clinical determination of oestrogen receptor protein levels, the specimens were considered appropriate for the reported studies. Median follow-up in patients still alive at the time of analysis was 5.5 years for both studies. Follow-up data were obtained regularly according to local guidelines. Ethical approval for the study was obtained from local ethics committees of each participating centre.

2.2. Determination of ER and PR expression

All tumours were ER and/or PgR positive (either $\geqslant$10 fmol/mg of cytosolic protein by enzyme immunoassay (EIA) or dextrane charcoal assay (DCC), or immunohistochemical Remmele Score > 0 on a scale ranging from 0 to 12).