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# Prognostic impact of thymidine phosphorylase expression in breast cancer – Comparison of microarray and immunohistochemical data

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ABSTRACT

Contrary findings exist according to the prognostic and predictive impact of thymidine phosphorylase (TP) expression in breast cancer. Goal of our study was to investigate TP expression on the mRNA level by microarray analysis in a large cohort of 1781 breast cancers and to analyse its prognostic impact. Furthermore we compared mRNA expression and immunohistochemical data to explain discrepancies between different studies.

The prognostic value of TP mRNA expression was analysed among $n = 622$ untreated patients. Strong expression in the subgroup of $n = 213$ ER-negative cancer correlates with improved survival ($P = 0.012$). In contrast, no difference in survival was detected in the ER-positive group. We also failed to observe a prognostic value of TP mRNA among $n = 435$ endocrine-treated patients as well as $n = 111$ CMF-treated patients.

In an unsupervised analysis, TP clustered together with genes expressed in immune cells. Moreover, among normal tissues the highest TP mRNA expression was found in tissues of the immune system. The profile of TP expression in breast cancers correlates to a metagene of interferon induction whereas the expression of TP among normal tissues correlates to a metagene for macrophages. When comparing microarray data with immunohistochemistry from the same $n = 51$ samples, there was no correlation with stained carcinoma cells. In contrast, the correlation with stromal staining was highly significant ($P < 0.001$). Thus TP mRNA from microarray mainly reflects expression in stromal and immune cells. This could account for discrepant results from mRNA and IHC studies.

In conclusion, the tumour infiltrating immune cells seem to be a major source of TP expression and predict a favourable prognosis in ER-negative breast cancer. Our data point to a role of TP in host immune response.



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## 1. Introduction

Thymidine phosphorylase (TP) catalyses the phosphorylation of thymidine and 2′-deoxyuridine to their respective bases and 2-α-deoxyribose-1-phosphate.[1] The enzyme is also known as platelet-derived endothelial cell growth factor (PD-ECGF) and has been reported to play a role in tumour growth and invasion. TP expression in various solid tumours is elevated compared to that in the adjacent non-neoplastic tissue components.[2] Furthermore the protein seems to have angiogenic properties but the precise mechanisms through which it promotes neoangiogenesis are still not fully elucidated. Mechanistically these properties suggest that a high TP expression may rather predict a poor outcome (reviewed in 3). On the other hand, this enzyme has been studied for its role in the treatment with fluoropyrimidine-containing chemotherapy. TP is involved in the conversion of 5FU to FdUMP, which finally leads to DNA damage. Therefore, a high TP expression may predict a good response to treatment. Because of this dual role of TP it is difficult to assess the contradictory results regarding a prognostic and/or predictive effect of this enzyme in different studies of 5FU-containing chemotherapy (reviewed in 3). A more simple case might be the treatment with capecitabine since TP seems to be a rate-limiting factor in one of the steps in the capecitabine pathway (conversion of 5′-DFUR to the active compound 5FU).[4] Capecitabine was actually designed to take advantage of the increased levels of TP observed in tumours as opposed to normal tissues, potentially allowing for selective toxicity in tumours.[5] However, even for the response to capecitabine treatment conflicting results were obtained regarding a positive predictive value of TP expression.[6–10] Some of these contrary findings could also be related to different applied methodologies. E.g. in breast cancer several immunohistochemical studies observed a benefit for patients with high TP expression[11–13] while other authors analysing mRNA expression failed to detect differences in prognosis.[14,15]

The aim of our study was to investigate the TP expression on the mRNA level in a large cohort of 1781 breast cancers and to analyse its prognostic impact. Furthermore we compared mRNA expression and immunohistochemical data to explain discrepancies between different studies.

## 2. Materials and methods

### 2.1. *Microarray data analysis*

A large series of Affymetrix U133A microarray datasets from several studies including a total of 1781 primary breast cancer samples was assembled as we have previously described.[16]

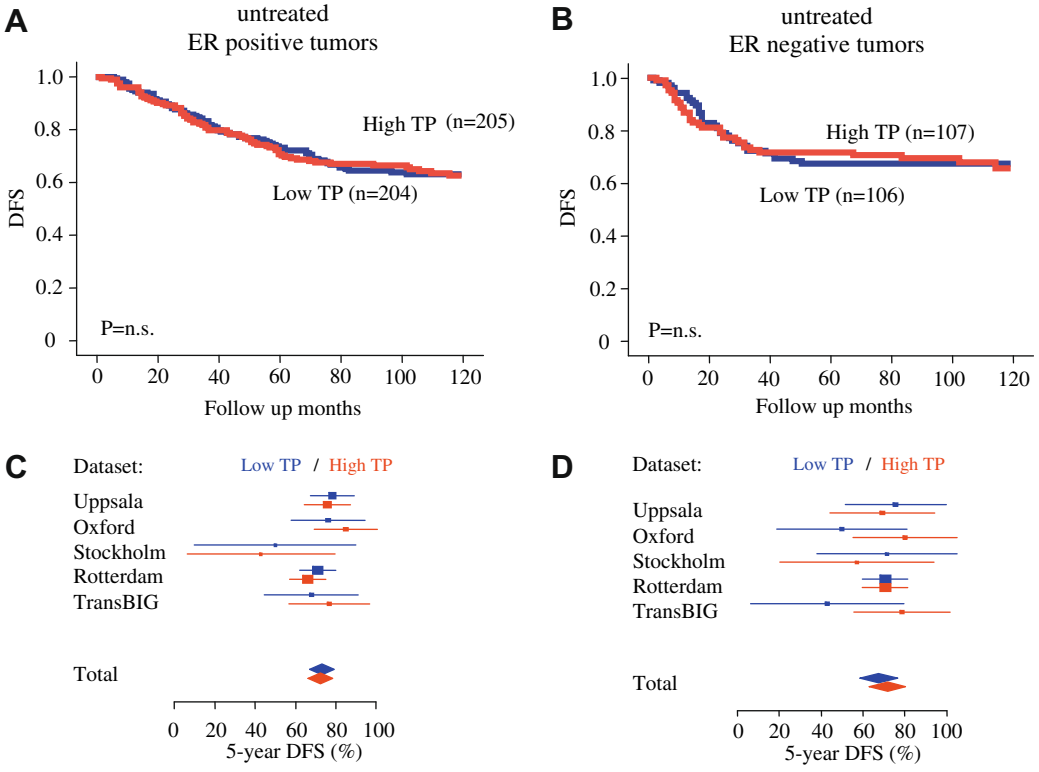


**Fig. 1 – Prognostic value of thymidine phosphorylase mRNA expression in breast cancer without systemic treatment. Kaplan–Meier analyses of disease-free survival (DFS) according to thymidine phosphorylase (TP) mRNA expression from microarray are presented separately for patients with ER-positive (A) and ER-negative (B) tumours. Forest plots for 5-year DFS rate estimates in the individual datasets are given in (C) for ER-positive samples and (D) for ER-negative samples. Box sizes correspond to the number of patients in the respective dataset and line length represent the standard error. All graphs are shown for median splits according to TP expression (blue: low TP; red: high TP). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**