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EUROPEAN JOURNAL OF CANCER 46 (2010) 549–557

available at www.sciencedirect.com

journal homepage: www.ejconline.com

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

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A R T I C L E I N F O

Article history:
Received 29 October 2009
Received in revised form 17 November 2009
Accepted 24 November 2009
Available online 18 December 2009

Keywords:
Breast cancer
Thymidine phosphorylase
Microarray
Immunohistochemistry
Prognosis

A B S T R A C T

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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doi:10.1016/j.ejca.2009.11.020

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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]

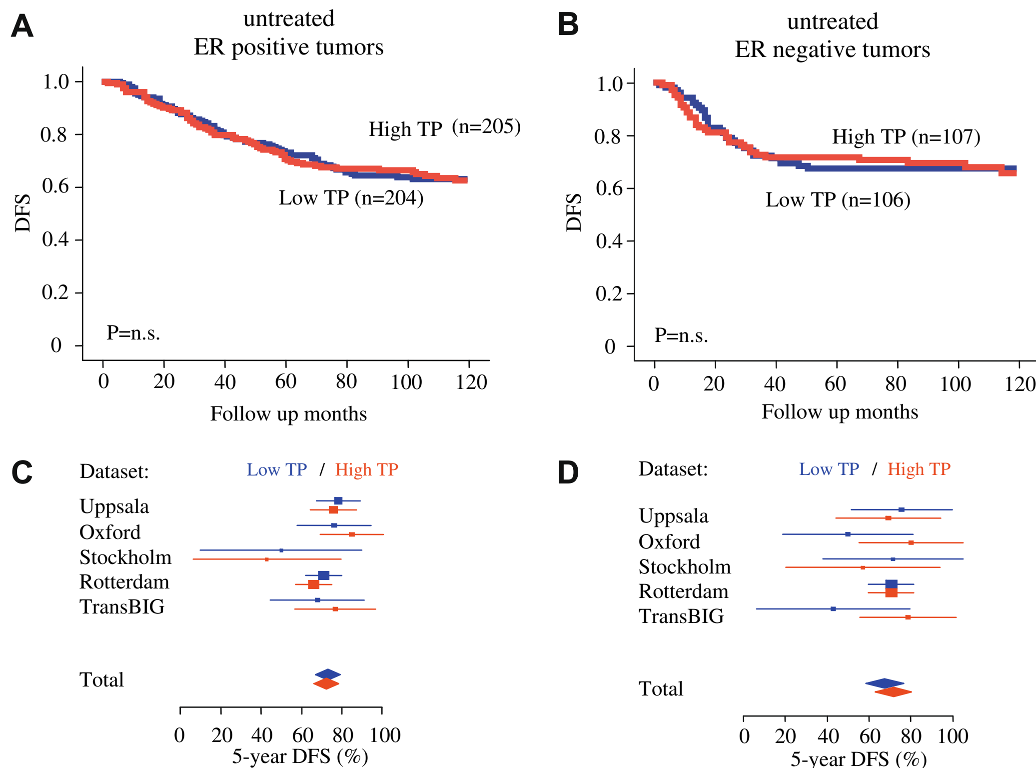

Fig. 1 – Prognostic value of thymidine phosphorylase mRNA expression in breast cancer without systemic treatment. Kaplan–Meier analyses of disease-free survival (DFS) according to thymidine phosphorylase (TP) mRNA expression from microarray are presented separately for patients with ER-positive (A) and 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.)

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Datasets from the following studies were included: Uppsala,[17] Oxford-Untreated,[18] Stockholm,[19] New York,[20] London,[21] Rotterdam,[22] Oxford-Tamoxifen and Villejuif,[23] ExpO,[24] MDA133,[25] and Frankfurt-1/2.[26–28] Characteristics of the individual datasets are presented in Supplementary Table 1. ER, PgR and HER2/neu status were determined from microarray using cutoffs derived from fitting two distributions to the data as we have recently described.[29] Two different Affymetrix Probesets for TP are available on the HGU133A microarray (204858_s_at and 217497_at). Since Probeset 217497_at displayed only weak signal intensities (see Supplementary Fig. S1) and we observed poor concordance with Probesets 204858_s_at especially for lower expression values, the Probeset 204858_s_at was selected for analyses. Microarray data from benign breast samples were obtained from Chen et al.[30]

2.2. *Comparison of microarray data and immunohistochemistry of breast cancer samples from the Frankfurt cohort*

Tissue samples of invasive breast cancer cases were obtained with institutional review board approval and informed consent from consecutive patients undergoing surgical treatment between December 1996 and July 2007 at the Department of Gynecology and Obstetrics at the Goethe-University in Frankfurt/M. Tissue samples from the same tumour were stored both in liquid nitrogen and in formalin-fixed paraffin-embedded blocks. Samples were characterised according to standard pathology including the oestrogen receptor status by ligand-binding assays or immunohistochemistry (IHC). Isolation of RNA and expression profiling using Affymetrix Human Genome U133A microarrays were performed as described elsewhere.[26,28] Specimens from the *Frankfurt* cohort with high or low thymidine phosphorylase mRNA expression, respectively, were identified and further investigated using immunohistochemistry. Paraffin sections (1 μm) were mounted on Superfrost Plus® slides, dewaxed in xylene and rehydrated through graduated ethanol to water. Antigens were retrieved in a pressure cooker (10 mM citrate buffer; pH 6.0; for 35 s). We used a monoclonal anti-TP antibody (clone PG44c, 1:100 diluted in antibody dilution buffer from DCS-Diagnostics, Hamburg, Germany).[11] Sections were incubated with antibodies for 1 h at room temperature. For negative controls, the primary antibodies were replaced with PBS. For detection, the Dako REAL EnVision® Detection System Peroxidase/DAB+ Rabbit/Mouse (Dako, Danmark) was used following the protocol of the supplier and sections were counterstained with haematoxylin solution, Gill No. 3 (Sigma). Expression levels were scored semi-quantitatively based on staining intensity and were classified as negative, weak, moderate and strong. Additionally, the percentage of positive cells was assigned stepwise in 5% steps. Negative control slides without antibody were included for each staining. Stained tumour tissues were scored blindly with respect to clinical and gene expression data.

2.3. *Statistical analysis*

Survival intervals were measured from the time of surgery to the time of death from disease or of the first clinical or radiographic evidence of disease recurrence. Data for women in whom the envisaged end point was not reached were censored as of the last follow-up date or at 120 months. We constructed Kaplan–Meier curves of disease-free survival and used the log rank test to determine the univariate significance of the variables. The Jonckheere-Terpstra-Test was used to determine if a high lymphocyte infiltration (LI) score correlates with higher thymidine phosphorylase expression. *P* values of less than 0.05 were considered to indicate a significant result and all reported P values are two-sided.

3. Results

3.1. *Prognostic value of TP mRNA expression in untreated and adjuvant-treated breast cancer patients*

To analyse the prognostic value of thymidine phosphorylase mRNA expression, we used a database of Affymetrix

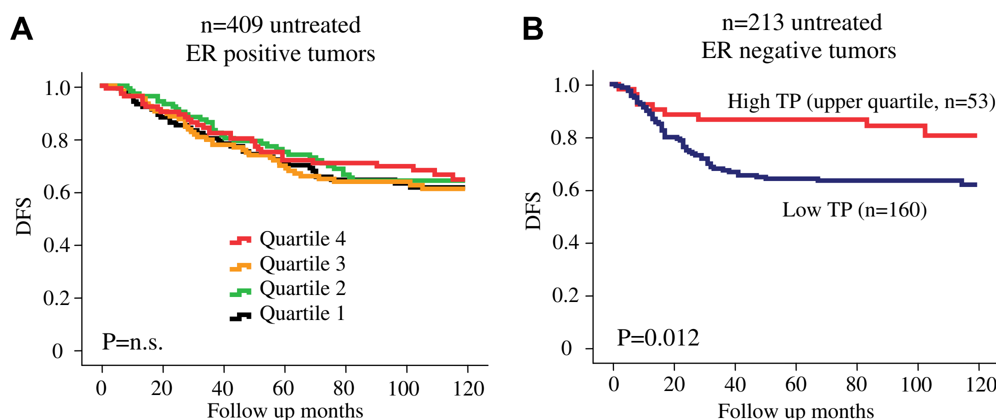

Fig. 2 – Prognostic value of the highest TP mRNA expression in ER-negative breast cancers. Samples were stratified into four quartiles according to TP expression. Kaplan–Meier analysis of disease-free survival among *n* = 622 untreated patients is shown. No significant difference in prognosis was observed among ER-positive tumours (A) while among ER-negative cancers (B) those samples with the highest TP mRNA expression (upper quartile) displayed a significant better prognosis than the rest of the samples (P = 0.012).

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microarrays of $n = 1781$ primary breast cancer samples which we have previously described.[16,29,31] For $n = 1263$ of the 1781 patients follow-up data were available. A detailed listing of the contributing datasets is given in Supplementary Table 1. To analyse the pure prognostic effect of TP expression only the patients without treatment are recommended. Six hundred and twenty-two of the patients with follow-up data did not receive any adjuvant treatment, $n = 409$ of them were ER positive and $n = 213$ ER negative. Kaplan–Meier analysis of disease-free survival (DFS) was performed separately for the ER-positive and ER-negative breast cancers to avoid a confounding effect of these different breast cancer subtypes. As shown in Fig. 1, we observed no significant difference in DFS when a median split according to expression values of thymidine phosphorylase was applied.

Since TP is involved in the conversion of 5-fluorouracile (5FU), high expression may be predictive for response to 5-FU-containing therapy. Hence, to evaluate a possible predictive effect of TP, we next analysed patients which were treated with CMF (cyclophosphamide/methotrexate/5FU) containing chemotherapy, though it is important to note that these patients also received other drugs. A total of $n = 111$ patients of this type were available. No difference in survival among these patients according to median TP expression was observed ($P = 0.65$ for $n = 70$ ER positive; $P = 0.88$ for $n = 41$ ER negative; $P = 0.86$ for all; not shown).

To more precisely assess the clinical importance of TP expression, we additionally stratified ER-positive and ER-negative tumours into four quartiles according to TP mRNA expression, respectively. Kaplan–Meier analyses of disease-free survival of the different subgroups were performed among the $n = 622$ patients with no adjuvant treatment. As shown in Fig. 2A, we observed no significant difference in prognosis according to quartiles of TP mRNA expression among the $n = 409$ untreated ER-positive tumours. In contrast, among the $n = 213$ ER-negative cancers the samples with the highest TP mRNA expression (upper quartile) displayed a significant better prognosis than the rest of the samples

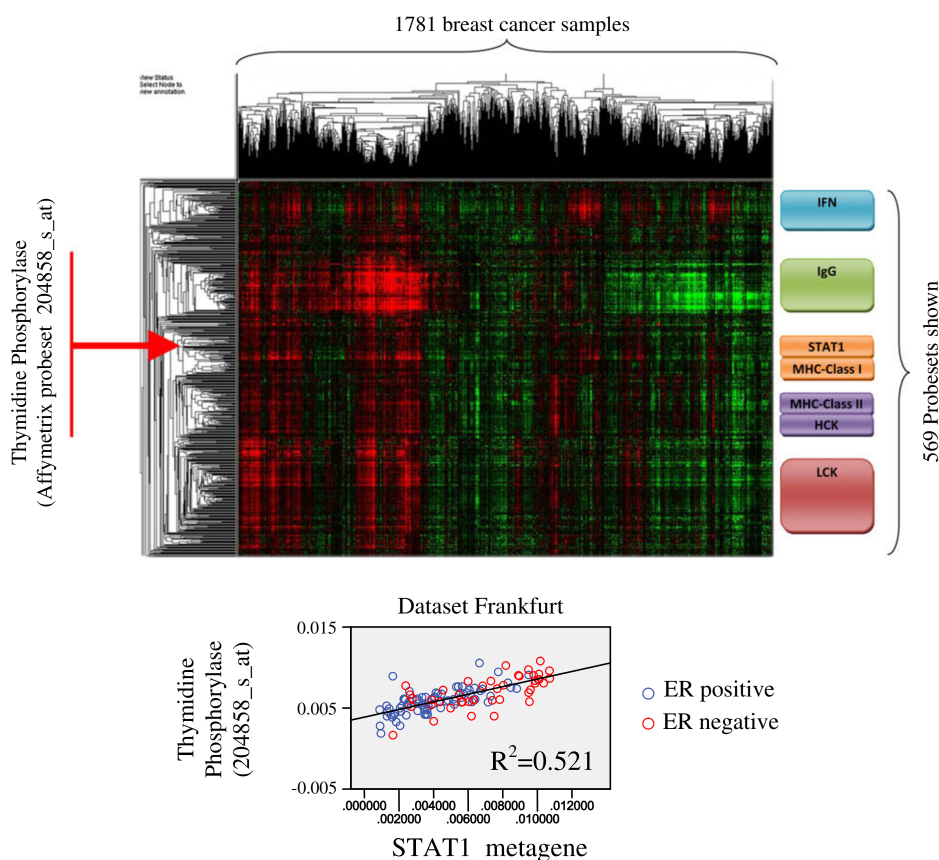

Fig. 3 – Correlation of thymidine phosphorylase mRNA expression with genes expressed in cells from the immune system. (A) Unsupervised clustering result of microarray data of breast cancer samples. Thymidine phosphorylase (red arrow) is found in a large cluster of about 569 genes with functions in immune cells which are displayed in the figure. Several subclusters from this large gene cluster can be attributed to different types of immune cells as we have recently described. Metagenes corresponding to these subclusters are represented by coloured boxes on the right. (B) Correlation of thymidine phosphorylase mRNA expression and the STAT1 metagene corresponding to interferon-induced genes among samples from the Frankfurt dataset. ER-positive and ER-negative samples are represented by blue and red circles, respectively (similar results from other datasets are shown in Supplementary Fig. S2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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(P = 0.012, Fig. 2B). The number of samples with adjuvant CMF treatment was too small to evaluate the predictive value of TP using quartile splits.

The observed results on the prognostic value of thymidine phosphorylase are in contrast to previous reports of a poor prognosis of tumours with high TP expression in cancer cells[32–36] as well as a predictive value of TP for CMF treatment.[11,12] One possible explanation for these discrepancies might be confounding effects of different cell types contributing to the mRNA expression of thymidine phosphorylase in the bulk tumour sample. Immunohistochemical studies have indicated the expression of TP besides carcinoma cells in several other stromal cell types such as fibroblasts, macrophages, endothelial cells and lymphocytes.[32,37,38]

3.2. *TP mRNA expression in breast cancers correlates with markers of immune cells*

To elucidate the source of TP mRNA expression we performed an unsupervised clustering of all 22,283 genes from the microarrays in our breast cancer dataset. Thymidine phosphorylase clustered together with a large set of about 600 genes with functions in immune cells as shown in Fig. 3A. We recently described the '*immune cell gene cluster*' and developed seven metagenes (IFN, IgG, STAT1, MHC-Class-I, MHC-Class-II, HCK and LCK) that correlate with the amount of different types of infiltrating immune cells in breast cancer.[16] We observed the strongest correlation of thymidine phosphorylase with the STAT1 metagene (exemplified for dataset Frankfurt in Fig. 3B and for additional datasets in Supplementary Fig. S2) which represents a series of interferon-inducible genes.

Moreover, the TP expression was also commonly associated with immune cells when analysing 79 non-malignant tissues using publicly available microarray data.[39] Among the normal samples with the highest TP expression more than half (8/15) corresponded to cells and tissues of the immune system (monocytes, myeloid cells, dendritic cells, NK cells, whole blood, tonsil, lymph node, thymus; see Supplementary Fig. S4). The highest expression of TP was observed in cells of the myeloid lineage like monocytes/macrophages and dendritic cells (Supplementary Fig. S5). In Fig. 4, the expression of the immune cell metagenes is compared to the TP profile among 44 immune system-related tissues. In this analysis, TP displayed the highest concordance to the HCK metagene. This metagene represents a variety of markers specific for macrophages and monocytes.[16] These results of TP mRNA expression among normal tissues were also validated on the protein level with immunohistochemical tissue microarray (TMA) data from the *Human Antibody Initiative* (http://www.proteinatlas.org[40]) (data not shown).

We next analysed, if the expression of thymidine phosphorylase detected by microarray correlated with the lymphocyte infiltration (LI) score of tumours as detected by the microscopic evaluation. We used two independent microarray datasets from London and Villejuif which are publicly available (GSE7390)[23] encompassing $n = 80$ samples with information on the LI score. A trend for a positive correlation of thymidine phosphorylase expression detected by microarray with the lymphocyte infiltration score was observed ($P = 0.055$, Jonckheere-Terpstra-Test; Supplementary Fig. S3). Taken together our results suggest that the mRNA expression signal of thymidine phosphorylase detected by microarray in breast cancer samples could originate from infiltrating im-

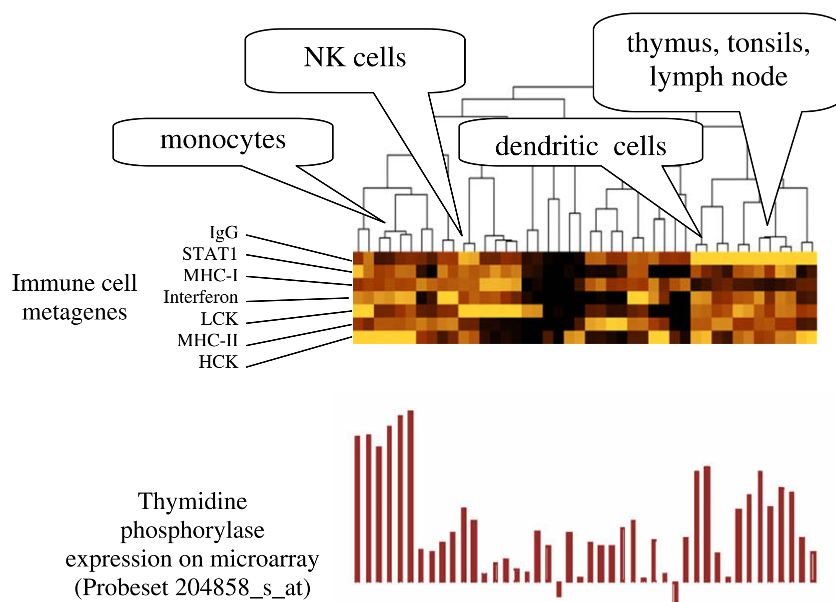

Fig. 4 – Comparison of immune cell metagenes and TP expression profile among immune system related tissues. Expression profiles of seven immune cell metagenes (A) are compared to the expression profile of thymidine phosphorylase (B) among 44 tissues related to the immune system. TP displayed the highest concordance to the HCK metagene. This metagene represents a variety of markers specific for macrophages, monocytes and other cells of the myeloid lineage of blood cells. Details on samples are given in Supplementary Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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mune cells. Alternatively the infiltrating immune cells might also induce thymidine phosphorylase expression in the carcinoma cells themselves.

3.3. *Comparison of TP mRNA expression and immunohistochemistry in breast cancers*

To compare microarray-based mRNA expression and immunohistochemistry data, we analysed $n = 51$ samples from our *Frankfurt* cohort using a monoclonal antibody against TP.[11] The samples were blindly analysed by immunohistochemistry and the percentage of stained carcinoma cells was recorded. As shown in Fig. 5A, strong cytoplasmic and nuclear TP staining of tumour cells were observed in some samples. In contrast, in other samples tumour cells showed no staining for TP (Fig. 5B). However, in all specimens we observed a stromal staining to a variable degree in macrophages as well as in endothelial cells and fibroblasts (Fig. 5B and C). In scatter plot analysis (Fig. 6), we observed no correlation when comparing mRNA expression from microarray with the percentage of stained carcinoma cells based on immunohistochemistry. On the other hand, samples with a strong stromal staining (represented by red triangles in Fig. 6) often displayed high mRNA expression values on microarray. When dichotomising the samples into positive versus negative based on the percentage of stained tumour cells, no significant difference in mRNA expression was observed (Mann–Whitney *U* test; Supplementary Fig. S6B). In contrast, a highly significant difference ($P < 0.001$; Supplementary Fig. S6C) was observed between those samples with strong or weak stromal staining for TP. These results suggest that stromal cells in the tumour seem to be the major source of mRNA expression detected by microarray.

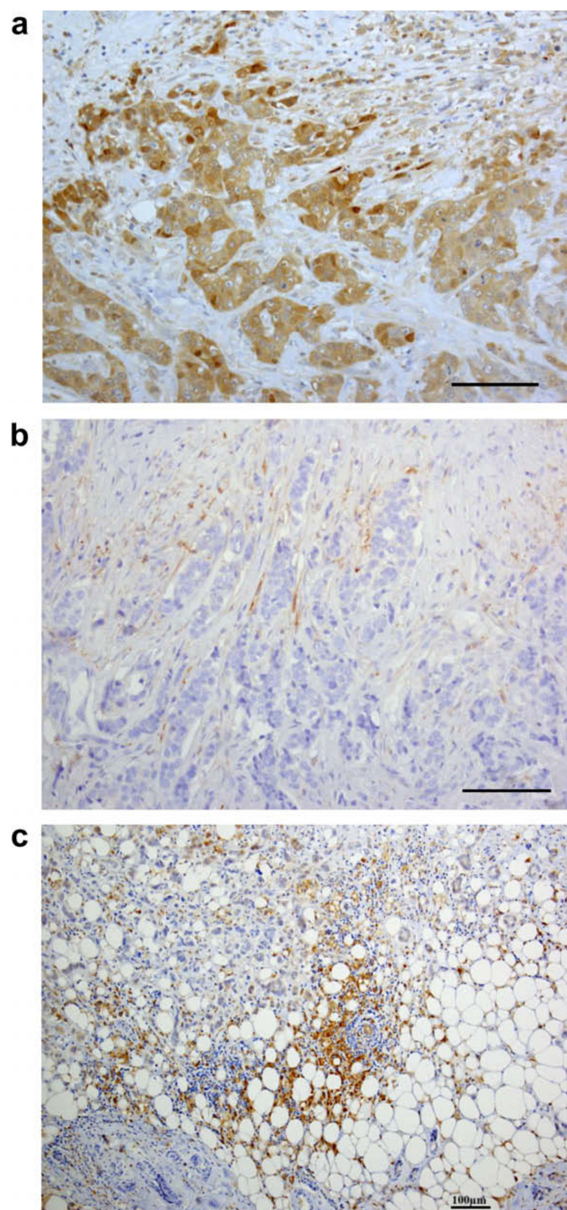

Fig. 5 – Immunohistochemical analysis of TP expression in breast cancer samples. (A) Sample with intensive cytoplasmic and nuclear staining of tumour cells as well as stromal staining. (B) Sample without staining of tumour cells but moderate staining of stroma (fibroblasts, macrophages and endothelial cells). (c) Sample with weak staining of tumour cells but strong staining of tumour-associated macrophages. Scale bars for all three pictures are 100 μm.

4. Discussion

A dual role for thymidin phosphorylase (TP) in cancer has been supposed.[1] On the one hand TP might stimulate tumor growth and on the other hand TP may play a role in the activation of fluoropyrimidine containing chemotherapy.[3] This may account for some of the conflicting results reported on the prognostic/predictive value of TP in breast and colon cancer.

When analysing microarray data from untreated breast cancer patients we failed to detect significant differences in prognosis using a median split according to TP mRNA expression. Similar results were obtained among $n = 435$ ER-positive patients which were treated solely with endocrine therapy (not shown). In a more detailed analysis applying quartiles for stratification a favourable prognosis only in the subgroup of ER-negative cancers of the samples with the highest TP expression was observed. These results are in contrast to previous reports of a poor prognosis of tumours with high TP expression in cancer cells.[32–36] Different methods of TP detection (mRNA versus IHC) might account for these discrepancies since other studies on the mRNA level also failed to detect differences in prognosis.[14,15] In addition to cancer cells we detected TP expression by IHC in stromal endothelial cells, monocytes and macrophages. This is in line with many other reports of TP expression in tumour stroma in breast cancer[2,10,11,13,41,42] and other tumour types.[32,37,38] Interestingly, some reports point to a favourable prognosis of cancers displaying strong TP expression in stromal cells like macrophages.[43,37]

In a previous study, we developed seven metagenes that can serve as markers for certain immune cell populations[16] in the tumour. In our breast cancer microarray dataset thymidine phosphorylase displayed the strongest correlation with the '*STAT1 metagene*' (exemplified for dataset *Frankfurt* in

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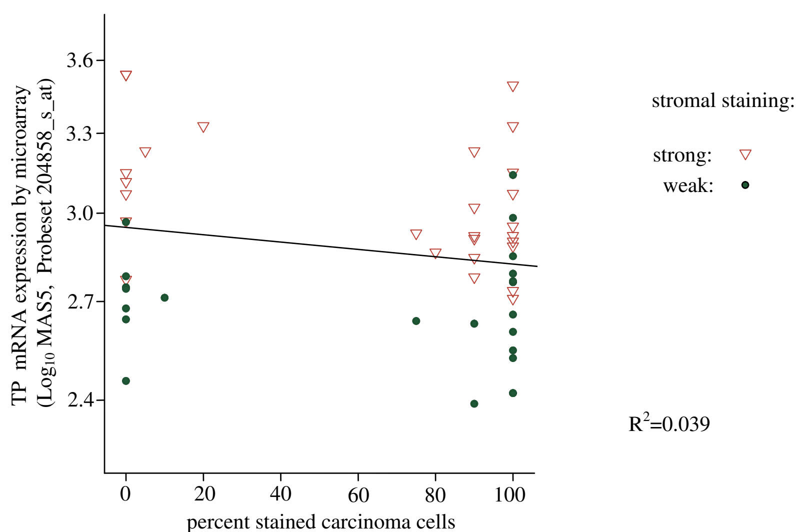

Fig. 6 – Correlation of TP mRNA expression from microarray and immunohistochemical staining of carcinoma cells. Shown is a scatter plot comparing the expression values of TP mRNA from microarray (probeset 204858_s_at) and the percentage of stained carcinoma cells from the same tumours using a monoclonal TP antibody. In addition, the samples were characterised according to weak (green dots) or strong (red triangles) stromal staining for TP. No correlation of mRNA expression with the percentage of stained carcinoma cells was observed but samples with strong stromal staining generally displayed high mRNA expression (see also Supplementary Fig. S6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3), which represents a series of interferon-inducible genes. Among normal immune system related tissues TP displayed the highest concordance to the '*HCK metagene*' (Fig. 4). This metagene represents a variety of markers specific for macrophages, monocytes and other cells of the myeloid lineage of blood cells. These data suggest a functional role of TP in different cells of the immune system and are supported by earlier immunohistochemical work.[38,44,45] When we compared TP microarray and IHC data from the same tumours we found no correlation of TP mRNA expression with the percentage of stained carcinoma cells (Supplementary Fig. S6B). In contrast, the difference between samples with strong or weak stromal staining was highly significant ($P < 0.001$; Supplementary Fig. S6C). All these results suggest that stromal cells seem to be the major source of TP mRNA expression detected by microarray analysis of bulk breast tumours. However it is important to note that the observed TP expression seem to result from stromal cells within the tumour and not from confounding benign tissue. As shown in Supplementary Fig. S7, the mRNA expression of TP in benign tissue is rather low. So for example different methods of biopsy collection should not account for the observed differences in TP mRNA expression.

We and others have recently shown that high amounts of stromal immune cells in tumours as detected by their specific metagene mRNA profiles as well as conventional methods identify patients with a good prognosis in ER-negative and HER2-positive cancers.[16,46–49] These data are highly important since all the available prognostic gene signatures do only work in the subset of ER-positive breast cancers.[50] Thus new prognostic markers were urgently needed for the ER-negative and HER2-positive subtypes. Moreover the presence of stromal immune cells even seems to allow the prediction of response to neoadjuvant chemotherapy.[16,51] TP mRNA expression by microarray strongly correlated with the '*immune cell gene cluster*' (Fig. 3). In line with this observation a favourable prognosis was observed for those samples with the highest TP expression among the untreated ER-negative tumours (Fig. 2B). Regarding a potential predictive value however the number of samples in this subgroups with adjuvant CMF treatment in our study was too small ($n = 3$ events) for a sound statistical analysis (not shown). In addition, there are no microarray data available from a cohort with capecitabine treatment which would be an important topic of future work.

In conclusion, our study demonstrates that mRNA from particular genes like TP can represent non-neoplastic cells and limit their prognostic and predictive utilities when measured in a microarray dataset as compared to immunohistochemical detection. On the other hand, these methods can allow precise quantitation and averaging over larger numbers of cells. Thus both techniques could complement each other in prospective studies on predictive biomarkers for capecitabine treatment.

Conflict of interest statement

None declared.

Acknowledgements

We thank Samira Adel and Katherina Kourtis for expert technical assistance. This work was supported by grants from the Deutsche Krebshilfe, the Margarete Bonifer-

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Stiftung, Bad Soden, the BANSS-Stiftung, Biedenkopf, the Werner Sack-Stiftung, Essen, and Dr. Robert Pfleger-Stiftung, Bamberg. These foundations had no role in planning of the study and writing of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2009.11.020.

REFERENCES

1. Bronckaers A, Gago F, Balzarini J, Liekens S. The dual role of thymidine phosphorylase in cancer development and chemotherapy. *Med Res Rev* 2009 [epub ahead of print].
2. Takebayashi Y, Yamada K, Miyadera K, et al. The activity and expression of thymidine phosphorylase in human solid tumours. *Eur J Cancer* 1996;**32A**:1227–32.
3. Koopman M, Venderbosch S, Nagtegaal ID, van Krieken JH, Punt CJ. A review on the use of molecular markers of cytotoxic therapy for colorectal cancer, what have we learned? *Eur J Cancer* 2009;**45**(11):1935–49.
4. Cao D, Russell RL, Zhang D, Leffert JJ, Pizzorno G. Uridine phosphorylase (–/–) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. *Cancer Res* 2002;**62**(8):2313–7.
5. Schüller J, Cassidy J, Dumont E, et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 2000;**45**(4):291–7.
6. Meropol NJ, Gold PJ, Diasio RB, et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006;**24**(25):4069–77.
7. Layman RM, Thomas DG, Griffith KA, et al. Neoadjuvant docetaxel and capecitabine and the use of thymidine phosphorylase as a predictive biomarker in breast cancer. *Clin Cancer Res* 2007;**13**(14):4092–7.
8. Puglisi F, Cardellino GG, Crivellari D, et al. Thymidine phosphorylase expression is associated with time to progression in patients receiving low-dose, docetaxel-modulated capecitabine for metastatic breast cancer. *Ann Oncol* 2008;**19**(9):1541–6.
9. Andreetta C, Puppin C, Minisini A, et al. Thymidine phosphorylase expression and benefit from capecitabine in patients with advanced breast cancer. *Ann Oncol* 2009;**20**(2):265–71.
10. Koopman M, Venderbosch S, van Tinteren H, et al. Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomised CAIRO study. *Eur J Cancer* 2009;**45**(11):1999–2006.
11. Fox SB, Engels K, Comley M, et al. Relationship of elevated tumour thymidine phosphorylase in node-positive breast carcinomas to the effects of adjuvant CMF. *Ann Oncol* 1997;**8**(3):271–5.
12. Yang Q, Barbareschi M, Mori I, et al. Prognostic value of thymidine phosphorylase expression in breast carcinoma. *Int J Cancer* 2002;**97**(4):512–7.
13. Ioachim E. Thymidine phoshorylase expression in breast cancer: the prognostic significance and its association with other angiogenesis related proteins and extracellular matrix components. *Histol Histopathol* 2008;**23**(2):187–96.
14. Relf M, LeJeune S, Scott PAE, et al. Expression of the angiogenic factor vascular endothelial cell growth factor, acidic and basic fibroblast growth factors, tumour growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res* 1997;**57**:963–9.
15. Li H, Suo Z, Zhang Y, et al. The prognostic significance of thymidine phosphorylase, thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expressions in breast carcinomas. *Histol Histopathol* 2004;**19**(1):129–36.
16. Rody A, Holtrich U, Pusztai L, et al. T-cell metagene predicts a favorable prognosis in estrogen receptor-negative and HER2-positive breast cancers. *Breast Cancer Res* 2009;**11**(2):R15.
17. Miller LD, Smeds J, George J, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci USA* 2005;**102**(38):13550–5.
18. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006;**98**(4):262–72.
19. Pawitan Y, Bjohle J, Amler L, et al. Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res* 2005;**7**(6):R953–64.
20. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature* 2005;**436**(7050):518–24.
21. Loi S, Haibe-Kains B, Desmedt C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol* 2007;**25**(10):1239–46.
22. Wang Y, Klijn JG, Zhang Y, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005;**365**(9460):671–9.
23. Desmedt C, Piette F, Loi S, et alTRANSBIG Consortium. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clin Cancer Res* 2007;**13**(11):3207–14.
24. The International Genomics Consortium (IGC). The expO project (Expression Project for Oncology) <http://www.intgen.org/>.
25. Hess KR, Anderson K, Symmans WF, et al. Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. *J Clin Oncol* 2006;**24**(26):4236–44.
26. Rody A, Holtrich U, Gaetje R, et al. Poor outcome in estrogen receptor-positive breast cancers predicted by loss of plexin B1. *Clin Cancer Res* 2007;**13**(4):1115–22.
27. Ahr A, Karn T, Solbach C, et al. Identification of high risk breast-cancer patients by gene expression profiling. *Lancet* 2002;**359**(9301):131–2.
28. Rody A, Karn T, Solbach C, et al. The erbB2+ cluster of the intrinsic gene set predicts tumor response of breast cancer patients receiving neoadjuvant chemotherapy with docetaxel, doxorubicin and cyclophosphamide within the GEPARTRIO trial. *Breast* 2007;**16**(3):235–40.
29. Karn T, Metzler D, Ruckhäberle E, et al. Data driven derivation of cutoffs from a pool of 3,030 Affymetrix arrays to stratify distinct clinical types of breast cancer. *Breast Cancer Res Treat* 2009 [epub ahead of print].
30. Chen DT, Nasir A, Culhane A, et al. Proliferative genes dominate malignancy-risk gene signature in histologically-normal breast tissue. *Breast Cancer Res Treat* 2009.

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31. Ruckhäberle E, Rody A, Engels K, et al. Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. *Breast Cancer Res Treat* 2008;**112**(1):41–52.
32. Imazano Y, Takebayashi Y, Nishiyama K, et al. Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma. *J Clin Oncol* 1997;**15**(7):2570–8.
33. Koukourakis MI, Giatromanolaki A, Kakolyris S, et al. Different patterns of stromal and cancer cell thymidine phosphorylase reactivity in non-small-cell lung cancer: impact on tumour neoangiogenesis and survival. *Br J Cancer* 1998;**77**(10):1696–703.
34. Takao S, Takebayashi Y, Che X, et al. Expression of thymidine phosphorylase is associated with a poor prognosis in patients with ductal adenocarcinoma of the pancreas. *Clin Cancer Res* 1998;**4**(7):1619–24.
35. van Triest B, Pinedo HM, Blaauwgeers JL, et al. Prognostic role of thymidylate synthase, thymidine phosphorylase/platelet-derived endothelial cell growth factor, and proliferation markers in colorectal cancer. *Clin Cancer Res* 2000;**6**(3):1063–72.
36. O'Byrne KJ, Koukourakis MI, Giatromanolaki A, et al. Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small-cell lung cancer. *Br J Cancer* 2000;**82**(8):1427–32.
37. Yasuno M, Mori T, Koike M, et al. Importance of thymidine phosphorylase expression in tumor stroma as a prognostic factor in patients with advanced colorectal carcinoma. *Oncol Rep* 2005;**13**(3):405–12.
38. Sivridis E, Giatromanolaki A, Papadopoulos I, et al. Thymidine phosphorylase expression in normal, hyperplastic and neoplastic prostates: correlation with tumour associated macrophages, infiltrating lymphocytes, and angiogenesis. *Br J Cancer* 2002;**86**(9):1465–71.
39. Su AI, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 2004;**101**(16):6062–7.
40. Berglund L, Björling E, Oksvold P, et al. A genecentric human protein atlas for expression profiles based on antibodies. *Mol Cell Proteomics* 2008(10):2019–27.
41. Kobayashi M, Sugimoto T, Okabayashi T, et al. Localization of thymidine phosphorylase in breast cancer tissue. *Med Mol Morphol* 2005;**38**:112–7.
42. Toi M, Ueno T, Matsumoto H, et al. Significance of thymidine phosphorylase as a marker of protumor monocytes in breast cancer. *Clin Cancer Res* 1999;**5**(5):1131–7.
43. Saito S, Tsuno N, Nagawa H, et al. Expression of platelet-derived endothelial cell growth factor correlates with good prognosis in patients with colorectal carcinoma. *Cancer* 2000;**88**(1):42–9.
44. Fox SB, Moghaddam A, Westwood M, et al. Plateletderived endothelial cell growth factor/thymidine phosphorylase expression in normal tissues: An immunohistochemical study. *J Pathol* 1995;**176**:183–90.
45. Yoshimura A, Kuwazuru Y, Furukawa T, et al. Purification and tissue distribution of human thymidine phosphorylase; high expression in lymphocytes, reticulocytes and tumors. *Biochim Biophys Acta* 1990;**1034**:107–13.
46. Teschendorff AE, Miremadi A, Pinder SE, Ellis IO, Caldas C. An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. *Genome Biol* 2007;**8**(8):R157.
47. Finak G, Bertos N, Pepin F, et al. Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med* 2008;**14**(5):518–27.
48. Calabrò A, Beissbarth T, Kuner R, et al. Effects of infiltrating lymphocytes and estrogen receptor on gene expression and prognosis in breast cancer. *Breast Cancer Res Treat* 2009;**116**(1):69–77.
49. Schmidt M, Böhm D, von Törne C, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. *Cancer Res* 2008;**68**(13):5405–13.
50. Wirapati P, Sotiriou C, Kunkel S, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res* 2008;**10**(4):R65.
51. Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neo-adjuvant chemotherapy in breast cancer. *J Clin Oncol* 2009. doi:10.1200/JCO.2009.23.7370.