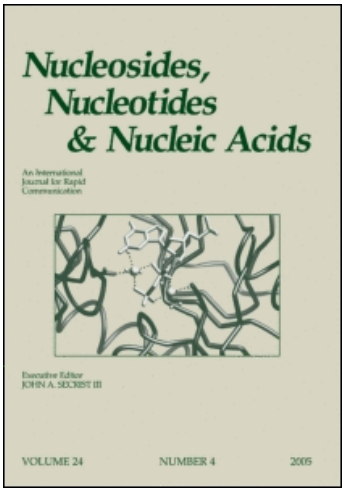

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Difference in Ki67 and Thymidylate Synthase Expression in Primary Tumour Compared with Metastatic Nodes in Breast Cancer Patients

A. Calascibetta[a]; D. Cabibi[b]; L. Rausa[c]; F. Aragona[b]; E. Barresi[b]; A. Martorana[b]; R. Sanguedolce[a]
[a] Dipartimento di Scienza Farmacologiche, Università degli Studi di Palermo, Palermo, Italy [b] Instituto di Anatomia Patologica, Università degli Studi di Palermo, Palermo, Italy [c] Dipartimento di Oncologia Sperimentale e Applicazioni Cliniche, Università degli Studi di Palermo, Palermo, Italy

To cite this Article Calascibetta, A. , Cabibi, D. , Rausa, L. , Aragona, F. , Barresi, E. , Martorana, A. and Sanguedolce, R.(2006) 'Difference in Ki67 and Thymidylate Synthase Expression in Primary Tumour Compared with Metastatic Nodes in Breast Cancer Patients', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1193 — 1196
To link to this Article: DOI: 10.1080/15257770600894527
URL: http://dx.doi.org/10.1080/15257770600894527

Nucleosides, Nucleotides, and Nucleic Acids, 25:1193–1196, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600894527

Taylor & Francis
Taylor & Francis Group

DIFFERENCE IN Ki67 AND THYMIDYLATE SYNTHASE EXPRESSION IN PRIMARY TUMOR COMPARED WITH METASTATIC NODES IN BREAST CANCER PATIENTS

A. Calascibetta □ *Dipartimento di Scienza Farmacologiche, Università degli Studi di Palermo, Palermo, Italy*

D. Cabibi □ *Instituto di Anatomia Patologica, Università degli Studi di Palermo, Palermo, Italy*

L. Rausa □ *Dipartimento di Oncologia Sperimentale e Applicazioni Cliniche, Università degli Studi di Palermo, Palermo, Italy*

F. Aragona, E. Barresi, and A. Martorana □ *Instituto di Anatomia Patologica, Università degli Studi di Palermo, Palermo, Italy*

R. Sanguedolce □ *Dipartimento di Scienze Farmacologiche, Università degli Studi di Palermo, Palermo, Italy*

□ *Breast cancer is a heterogeneous disease, so therapeutic predictive biological markers need to be identified. To date an accurate evaluation of predictive markers is mainly done at the primary site; however, the main goal of adjuvant therapy for breast cancer is the control of micrometastases. The aim of this study is to assess as therapeutic and/or prognostic marker, the proliferation status of primary tumors and involved nodes as measured by Ki67 and thymidylate synthase (TS) expression, in 30 breast cancer node positive patients. TS is the main target of 5-fluorouracil (5-FU) activity, and its overexpression is one of the mechanisms of 5-FU drug resistance; however, in some studies its absence is responsible for a worse response to 5-FU. Our results show that malignant cells of involved nodes were in a post mitotic phase of the cell cycle, and show a low proliferation index and TS expression, while the primary tumours and controls, were strongly positive. On these basis we can hypothesize that these cells could be less sensitive to 5-FU. Further studies are necessary to identify other mechanisms responsible for their metastasing capability and/or for their aggressiveness.*

Keywords Breast cancer; Thymidylate synthase; Proliferation index, Ki67

This work was partially supported by the fellow-ship entitled "Marilù Parlavecchio Comito" and by MIU 2004.

Address correspondence to A. Calascibetta, Dipartimento di Scienza Farmacologiche, Università degli Studi di Palermo, Via del Vespro 159, 90100, Palermo, Italy. E-mail: doctorjoe@inwind.it

INTRODUCTION

Breast cancer is a tumor characterized by a strong variability, therefore, therapeutic predictive biological markers need to be identified. Tumor cell proliferation has acquired relevance as an indicator of prognosis and of response to treatment.[1] Ki67 is one of the most frequently used markers to assess proliferative activity,[2] as well as thymidylate synthase (TS) is a marker of proliferation status, that it is related to the percentage of cells in S phase of the cell cycle.[3] TS is a critical target for the fluoropyrimidines, an important group of antineoplastic drug that are used widely in the treatment of breast cancer. Expression of TS is associated with response to 5-FU in human colorectal, gastric, and breast cancer; in fact many papers show high TS levels are associated with a worse prognosis,[4] but according to some literature, tumors showing low TS levels are resistant to 5-FU treatment.[5,6] Although much of the recent emphasis of prognostic testing in breast cancer patients mainly has been related to node negative status,[7] it could be useful to study clinical, prognostic, and predictive features of axillary metastases. Even though nodal status is one of the most important predictor of distant metastases and overall survival,[8] this factor is not always sufficient to allow treatment decision to be made; in fact for these patients, therapeutic options range from tamoxifen alone to several antineoplastic drug combination protocols. The aim of this study is to evaluate the Ki67 and TS expression as therapeutic and/or prognostic markers for the proliferation status of primary tumours compared to that of the involved nodes in 30 breast cancer node positive patients.

PATIENTS AND METHODS

Our study was performed using formalin-fixed paraffin-embedded primary tumour samples from 30 patients with metastatic lymph nodes at diagnosis and previously untreated. For each case we use four 3 μm sections that were dewaxed and rehydrated. Antigen retrieval was carried out using Dako (Denmark) antigen retrieval fluid and microwaving at full power for 30 minutes. Sections were then treated with hydrogen peroxidase for 5 minutes, followed by TS 106 at a diluition of 1:10 over night at 4°C. Visualization was obtained by Dako visualization system. The same protocol was used for the antibody Ki67 at a dilution 1:50 for 1 hour. As a positive control we used the lymphoid germinal centres and the normal glands of the sample when present near the tumour.

RESULTS

Twenty-two of our 30 samples of primary tumor showed a widespread positivity in more than 50% of neoplastic areas evaluated in 10 high power

TABLE 1 TS and Ki67 Levels in Primary Tumor and Involved Nodes of Breast Cancer Patients

	Metastatic Nodes TS +++/Ki67 +++	Metastatic Nodes TS +/− Ki67 +/−
Primary tumor TS +++/Ki67+++ (22)	6	16
Primary tumor TS +/−/Ki67+/− (8)	8	0
TOT 30	14	16

+++ = positivity >50% of neoplastic areas for TS; positivity >50% of nuclei for Ki67 +/− = positivity <10% of neoplastic areas for TS; positivity <10% of nuclei for Ki67.

fields (HPF) and were classified as positive for TS staining (+++). Ki67 also was present in more than 50% of nuclei, indicating a high proliferation index (+++). Of the 22 correspondent metastatic nodes, 16 showed a very low TS expression, in less then 10% of neoplastic areas and very low Ki67 positivity (+/−), while 6 metastatic nodes were strongly positive (+++) (see Table 1). There was no correlation with the clinicopathological features of the patients, because the node negativity was independent from staging, grading, and histotype of the tumor.

DISCUSSION

The search for prognostic factor of breast cancer has been largely focused on node-negative disease, but about 25% of these patients are destined to relapse, despite treatment.[7] By contrast, the majority of patients with node-positive breast cancer can be treated by adjuvant chemotherapy routinely. Prognostic factors in this group have received less attention, because of the definite benefit derived from adjuvant chemotherapy. In an analysis of many individual parameters, such as differentiation, histotype, nuclear pleomorphism, it has been shown that the presence of high proliferation activity is the strongest predictor of decreased survival.[9] Proliferation activity can be evaluated by Ki67 and TS expression. TS is the main target for 5-FU activity, a drug frequently used in the treatment of breast cancer patients. TS expression has been associated with clinical outcome in breast cancer; previous studies have shown that tumours showing high TS contents do not respond to 5-FU.[4] However , many patients with low TS expression also fail to respond to treatment.[5] Our study showed that a group of breast primary carcinomas have a high TS and Ki67 expression, while their lymph node metastases have a very poor expression of TS and Ki67 suggesting a low proliferative activity. This was unexpected due to the well known aggressiveness of metastases, thus, mechanisms other than proliferation could be responsible for the invasion by these cells. On these basis, we can hypothesize that since nonreplicating metastatic node cells have low TS expression, they could be less sensitive to 5-FU, because the target is lacking. We underline the necessity to study the expression of these prognostic

markers in both primary and metastatic tumors, because differences in the expression of the proliferating index, could be a marker able to identify possible drug resistant tumor cells.

REFERENCES

1. Keshgegian, A.A.; Cnaan, A. Proliferation markers in breast carcinoma: mitotic figure count, S-phase fraction, proliferation cell nuclear antigen, Ki 67 and Mib1. *Am. J. Clin. Pathol.* **1995**, 104, 42–49.
2. Isola, J.J.; Helin, H.J.; Helle, M.J.; Kallioniemi, O.P. Evaluation of cell proliferation in breast carcinoma: comparison of Ki 67 immunohystochemical study, DNA flow cytometric analysis and mitotic count. *Cancer* **1990**, 65, 1180–1184.
3. Nishimura, R.; Nagao, K.; Miyayama, H.; et al. Thymidylate syntahse levels as therapeutic and prognostic predictor in breast cancer. *Anticancer. Res.* **1999**, 19, 5621–5626.
4. Kakimoto, M.; Uetake, H.; Osanai, T.; et al. Thymidylate synthase and dihydropyrimidine dehydrogenase gene expression in breast cancer predicts 5 FU sensitivity by a histocultural drug sensitivity test. *Cancer Lett.* **2005**, 223, 103–111.
5. Cabibi, D.; Calascibetta, A.; Campione, M.; Barresi, E.; Rausa, L.; Dardanoni, G.; Aragona, F.; Sanguedolce R. Clinical relevance of thymidylate synthase expression in the signet ring cell hystotype of colorectal carcinoma. *Eur. J. Cancer* **2004**.
6. Foekens, J.A.; Romain, S.; Look, M.P.; Martin, P.M.; Klijn, J.G. Thymidine kinase and thimidylate synthase in advanced breast cancer: response to tamoxifene and chemotherapy. *Cancer Res.* **2001**, 61, 1421–1425.
7. McGuire, W.L.; Clark, G.M. Prognostic factors and treatment decisions in axillary- node-negative breast cancer. *N. Engl. J. Med.* **1992**, 326, 1756–1761.
8. Van Diest, P.J.; Baak, J.P.; Matze-Cok, P.; et al. Prediction of response to adjuvant chemotherapy in premenopausal lymph-node positive breast cancer patients with morphometry, DNA flow cytometry and Her-2/neu oncoprotein expression: Preliminary results. *Pathol. Res. Pract.* **1992**, 188, 344–349.
9. Perrone, F.; Carlomagno, C.; Lauria, R.; De Lauentis, M.; Marabilo, A.; Panico, L.; Pettinato, G.; Petrella, G.; Gallo, C.; Bianco, A.R.; Di Placido, S. Selecting high risk early breast cancer patients: whath to add to the number of metastatic nodes? *Eur. J. Cancer* **1996**.