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Circulating tumour cells in breast cancer at the diagnosis are associated with lymph node involvement, tumour size and negative ER status

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Abstract

We investigated the association of circulating tumour cells (CTC) with poor prognostic markers in breast cancer (BC) at time of diagnosis. Peripheral blood (PB) samples from 190 patients with invasive BC, 12 patients with in situ BC, before therapy and/or surgery, and 330 from patients without BC were tested for CTCs by RT-PCR for human mammaglobin (hMAM). hMAM was expressed only in PB of invasive BC (9.5%) and a significant correlation was found between CTCs with lymph node involvement, tumour size and negative ER. We conclude that CTC detection in invasive BC may be an additional poor prognostic indicator.

Introduction

Breast cancer (BC) has been shown to shed malignant cells in peripheral blood (PB) at the earliest stages of primary development [1]. Although numerous studies suggest the promising role of CTCs in routine management of BC patients, their clinical implication is still under investigation [1]. In the present study we investigated the association of CTCs, indirectly detected by hMAM gene expression, with tumour type and size, grading, lymph node involvement, ER and PGR status, HER-2/neu expression and Ki-67 labelling index at time of diagnosis [2].

Materials and Methods

The study was approved by the Institutional Ethics Committee. BC cells were separated by density gradient centrifugation from 5 ml of peripheral blood (PB) drawn in EDTA. Immunohistochemistry, RNA extraction, PCR primers, RT-PCR reaction condition and statistical analysis were conducted as previously described [2] (fig. 1).

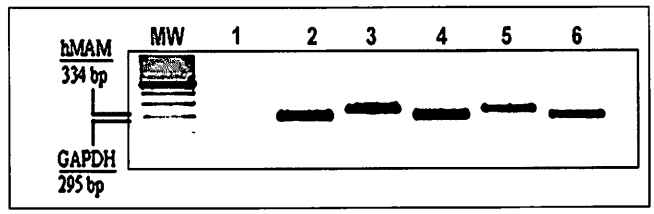

Figure 1. Ethidium bromide-stained 1.5% agarose gel of RT-PCR amplified hMAM. Lane 1: benign breast disease; Lane 3: breast cancer; Lane 5: breast cancer cell line MDA-MB415. Lane 2,4,6: GAPDH. MW, molecular weight marker.

Results

HMAM expression was analyzed in 190 patients with invasive BC at primary diagnosis, 12 patients with in situ BC and 330 without BC.
HMAM was detected in 18/190 (9.5%) PB samples from invasive BC but in none of those with in situ BC as well as in controls (not shown).
In multivariate analysis, direct association between CTC detection and prognostic factors was found for nodal status (positive *vs* negative, odd ratio=5.6, *P*=0.009), increased tumour size (odd ratio=2.3, *P*=0.207), ER status (negative *vs* positive, odd ratio=2.5, *P*=0.227) (tab. 1).

Prognostic factor		Multivariate analysis		
		Odd Ratio	*95% CL*	*P*
Tumour size	≤ 2 (pT1) *vs* >2 (pT2-pT4)	2.3	0.6-9.0	0.207
Nodal status	pN0 vs pN1-pN3	5.6	1.4-22.6	0.009
ER	Positive vs Negative	2.5	0.6-10.0	0.227

Table 1. Correlation between CTC in PB and prognostic factors.

Discussion

hMAM is a specific marker for CTC detection in PB [2]. However, the frequency of hMAM expression in PB and its association with poor prognostic markers from BC remains controversial [1]. Conflicting findings may

arise from the different methodologies applied and the incomplete understanding of CTC functional biology in BC [2]. The concordance of results represents the basis for CTCs before application in the routine clinical decision. In 2007, ASCO has included the CTC as tumour markers recommended in the guidelines for the use in BC [3]. We are in line with ASCO guidelines advicing that further research would be necessary to standardize the methodologies and to understand the CTC functional biology.

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