Mesenchymal phenotype of circulating tumor cells-enriched blood fraction and lymph node metastasis formation potential

Aleksandra Markiewicz, Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG & MUG

Supervisors: prof. dr hab. Jacek Bigda, dr Anna Żaczek

Introduction

Circulating tumor cells (CTCs) that present mesenchymal phenotypes can escape standard methods of isolation, thus limiting possibilities for their characterization. Whereas mesenchymal CTCs are considered to be more malignant than epithelial CTCs, factors responsible for this aggressiveness have not been thoroughly defined. This study analyzed the molecular profile related to metastasis formation potential of CTC-enriched blood fractions obtained by marker unbiased isolation from breast cancer patients without (N-) and with lymph nodes metastases (N+).

Materials and methods

Blood samples drawn from 117 patients with early-stage breast cancer were enriched for CTCs using density gradient centrifugation and negative selection with anti-CD45 covered magnetic particles. In the resulting CTC-enriched blood fractions, expression of CK19, MGB1, VIM, TWIST1, SNAIL, SLUG, HER2, CXCR4 and uPAR was analyzed with qPCR. Results were correlated with patients’ clinicopathological data.

Results

CTCs (defined as expression of either CK19, MGB1 or HER2) were detected in 41% (20/49) of N- and 69% (34/49) of N+ patients (P = 0.004). CTC-enriched blood fractions of N+ patients were more frequently VIM (P = 0.02), SNAIL (P = 0.059) and uPAR-positive (P = 0.03). Positive VIM, CXCR4 and uPAR status correlated with >3 lymph nodes involved (P = 0.003, P = 0.01 and P = 0.045, respectively). In the multivariate logistic regression MGB1 and VIM-positivity were independently related to lymph node involvement with corresponding overall risk of 3.2 and 4.2. Moreover, mesenchymal CTC-enriched blood fractions (CK19/-VIM+ and MGB1+ or HER2+) had 4.88 and 7.85-times elevated expression of CXCR4 and uPAR, respectively, compared with epithelial CTC-enriched blood fractions (CK19+/VIM- and MGB1+ or HER2+).

Conclusions

Tumors of N+ patients have superior CTC-seeding and metastatic potential compared with N- patients. These differences can be attributed to VIM, uPAR and CXCR4 expression, which endow tumor cells with particularly malignant phenotypes.