ORIGINAL ARTICLE

CXCL12 and *ADAM23* hypermethylation are associated with advanced breast cancers

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More than 25% of the patients with breast cancer (BC) develop metastatic disease. In the present study, we investigated the relationship between DNA methylation levels in genes regulating cell growth, invasiveness, and metastasis and advanced BCs and evaluated the clinical utility of methylation profiles for detecting metastatic potential. Pyrosequencing was used to quantify methylation levels in 11 cancer-associated genes in primary tumors (PTs), lymph node metastases (LNMs), plasma (PL), and blood cells from 206 patients with invasive BC. Protein expression was evaluated using immunohistochemistry. PTs showed hypermethylation of A isoform of the RAS-association domain family 1 (*RASSF1A*), adenomatous polyposis coli (*APC*), chemokine C-X-C motif ligand 12 (*CXCL12*), and disintegrin and metalloprotease domain 23 (*ADAM23*) (means 38.98%, 24.84%, 12.04%, and 10.01%, respectively). Positive correlations were identified between methylations in PTs and LNMs, but not between PL and PTs. The cumulative methylation of PTs and LNMs manifested similar spectrums of methylated genes that indicate the maintaining of aberrant methylation during breast tumorigenesis. Significantly increased methylation levels in *RASSF1A*, *APC*, *CXCL12*, and *ADAM23* were found in estrogen receptor (ER) positive BCs in comparison with ER negative cases. Regarding these results, the evaluation of DNA methylation could be more informative in testing of patients with ER positive BC. The risk for LNMs development and higher proliferation of cancer cells measured through Ki-67 expression was increased by hypermethylation of *CXCL12* and *ADAM23*, respectively. Therefore, the quantification of *CXCL12* and *ADAM23* methylation could be useful for the prediction of advanced stage of BC. (Translational Research 2015;■:1–14)

Abbreviations: ADAM23 = disintegrin and metalloprotease domain 23; APC = adenomatous polyposis coli; BC = breast cancer; BRMS1 = breast cancer metastasis suppressor 1; CDH1 = cadherin 1, type 1; cfDNA = cell-free DNA; CMI = cumulative methylation index; CXCL12 =

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chemokine C-X-C motif ligand 12; DIC = ductal invasive carcinoma; ER = estrogen receptor; ERα = estrogen receptor alpha; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemical; IRS = immunoreactive score; Ki-67 = index of proliferative activity; LIC = lobular invasive carcinoma; LN = lymph node; LNM = lymph node metastasis; PBCs = peripheral blood cells; PL = plasma; PPs = percentage of positive cells; PR = progesterone receptor; PR-B = B isoform of the progesterone receptor; PT = primary tumor; RASSF1A = A isoform of the RAS-association domain family 1; SD = standard deviation; SI = staining intensity; SOCS1 = suppressor of cytokine signaling 1; SYK = spleen tyrosine kinase; TIMP3 = tissue inhibitor of the metalloproteinases 3; TNM = TNM classification

AT A GLANCE COMMENTARY

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Background

The aberrant methylation profiles in tumor tissues lead to the deregulation of transcription activities in many genes that strongly influence the cancer cell behavior including the invasivity and metastases forming. We investigated the relationship between DNA methylation levels in genes regulating cell growth, invasiveness, and metastasis and advanced breast cancers (BCs) and evaluated the clinical utility of the methylation profiles for detecting metastatic potential.

Translational Significance

The results of our study indicate that the methylation profiles of *RASSF1A*, *APC*, *CXCL12*, or *ADAM23* could be more informative in testing of patients with estrogen receptor positive BC, and the hypermethylation of *CXCL12* and *ADAM23* genes could be useful for the prediction of advanced stage of BC.

INTRODUCTION

Breast cancer (BC) is the most common malignancy in women and it represents 28.8% of all the female cancers diagnosed in 40 European states in the year 2012.[1] One of the primary causes of the high mortality is that more than 25% of patients with BC develop metastatic disease, and approximately 6% of the patients are diagnosed with metastatic disease at the time of the initial diagnosis.[2]

Metastasis development is a complex process that is defined by distinct steps involving the local invasion of cancer cells, their intravasation into adjacent vessels, transit through the circulatory system and evasion of the host immune system, extravasation into the parenchyma of distant organs, and the colonization and formation of micrometastases, followed by the proliferation and progression of macrometastases.[3]

The invasive behavior of cancer cells is associated with the presence of tumor DNA fragments in the peripheral blood of patients with cancer. In healthy human plasma (PL), the cell-free DNA (cfDNA) is derived from apoptotic cells with a primarily hematopoietic origin.[4,5] In patients with cancer, the apoptotic and necrotic cancer cells are the primary source of the tumor cfDNA.[6] Many studies have used cfDNA for the qualitative or quantitative evaluation of cancer-specific alterations, including changes in methylation profiles; however, the clinical utility of cfDNA has been critically re-evaluated because of the high methodical diversity and limited diagnostic sensitivity and specificity.[7]

Cancer cells intravasate into both blood and lymphatic vessels, but the hematogenous circulation is considered to be the major route for metastatic dissemination. To date, it is not clear, whether tumor cells actually metastasize from the lymph nodes (LNs) to the secondary organs, or whether the presence of tumor cells in the LNs only reflects their intrinsic invasiveness.[8] Regardless the molecular characters of lymph node metastasis (LNM) provide useful information for the development of more effective therapy.

Human cancer represents a heterogeneous group of diseases driven by progressive genetic and epigenetic alterations including the hyper- and hypomethylation of DNA and changed histone modifications that result in remodeling of the chromatin structure.[9] The aberrant methylation profiles in genes, which are responsible for specific processes in tumorigenesis, could be used as prognostic or predictive markers. Moreover, dynamic methylation changes during tumorigenesis modulate the presence of variable expression profiles in cancer cells that lead to different behaviors including sensitivity to therapy.

In our study, we were focused on the DNA methylation changes in genes that regulate cell growth and act in the inhibition of invasivity and metastasis processes. All evaluated genes have the cytosine-phosphate-guanine (CpG) islands in their promoter