PRECISION MEDICINE IN BREAST CANCER-EVALUATION OF MIDKINE (MDK) AS A POTENTIAL BIOMARKER

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Abstract

Midkine (MDK) is a secreted heparin-binding growth factor reportedly overexpressed in several malignancies, including breast cancer. We sought to validate the clinical value of MDK as a biomarker for breast cancer monitoring, prognosis and response to treatment. MDK was quantified in plasma samples from 100 Cypriot breast cancer patients (early-stage and metastatic) utilizing Enzyme-Linked Immunosorbent Assay (ELISA). MDK levels were subsequently associated with patient clinicopathological features. MDK was significantly higher within the metastatic compared to the early-stage population. In the metastatic setting, disease detection rate based on MDK overexpression was comparable to that of conventional tumor markers CEA and CA 15-3. MDK overexpression without parallel CEA or CA 15-3 upregulation was observed in ~10% of the metastatic patients, highlighting its potential use in disease detection/monitoring when other markers are not expressed. A correlation between changes in MDK plasma levels—during treatment—and response to treatment as assessed through imaging was suggested in metastatic patients. Overall, MDK shows promise as a potential breast cancer biomarker, but further validation is required for its establishment.

Introduction

Midkine (MDK) is a secreted heparin-binding growth factor, involved in pathways associated with cell growth, survival, migration and angiogenesis. Despite its involvement in several physiological processes, MDK overexpression has been linked with pathological conditions including several malignancies. Preliminary studies demonstrated increased MDK expression in breast cancer patients compared to healthy controls. Additionally, MDK levels were particularly high during disease progression into more advanced stages. Although the role of MDK in breast cancer development and progression is under ongoing investigation, MDK has gained attraction as a candidate biomarker for disease diagnosis, prognosis and monitoring. Here, we aimed at investigating the clinical value of MDK as a breast cancer biomarker for disease prognosis, monitoring and response to treatment. In this respect, plasma MDK levels were quantified in early-stage and metastatic breast cancer patients and were subsequently correlated with the clinicopathological features from the respective patients.

Materials and Methods

100 female breast cancer patients, 50 early-stage and 50 metastatic were included in this study (**Figure 1**). Blood was collected from patients both prior to receiving systemic therapy and on a follow-up basis. Follow up samples for early-stage patients were collected after systemic treatment, while for the metastatic patients at 3 months after the initiation of systemic treatment. Plasma MDK levels were measurement utilizing Enzyme-Linked Immunosorbent Assay (ELISA). All samples were tested in duplicates. MDK plasma concentration was calculated against a standard curve generated from known MDK concentrations. Aberrantly high MDK levels were determined based on published data reporting the normal range of MDK levels in healthy controls. Clinicopathological data were extracted from patients' medical records and used for correlation with MDK plasma levels to identify potential associations.

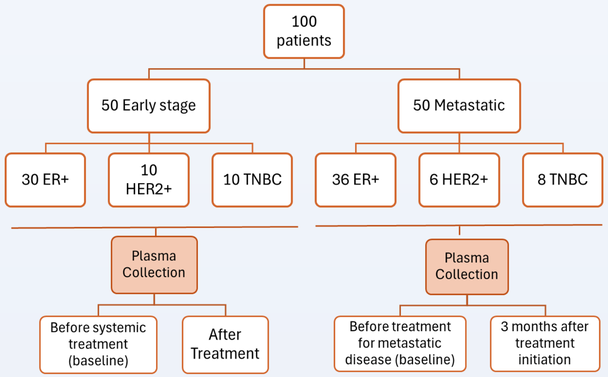

Figure 1: Study design. 50 early-stage and 50 metastatic patients were included in the study. Estrogen receptor+ (ER+), Human Epidermal Growth Factor+ (HER2+) and triple negative (TNBC) breast cancer cases were included.

Results

MDK levels were significantly higher in the metastatic compared to the early-stage setting—median levels 1363pg/mL for metastatic and 401.5pg/mL for early-stage (**Figure 2**). Comparison of the baseline and follow-up samples did not reveal significant differences in MDK levels at the two time points either in the early or the metastatic patients, although a trend for MDK level reduction at the 3-month follow-up was observed in the metastatic setting.

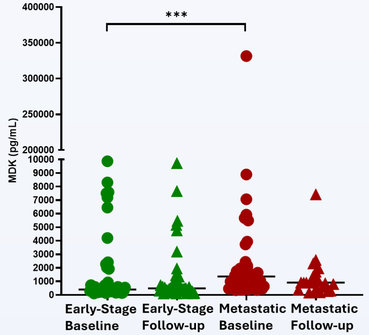

Figure 2: MDK levels in plasma samples from early-stage and metastatic breast cancer patients prior to initiation of systemic treatment (baseline) and on a follow-up basis. *** p=0.0002, Mann-Whitney test.

Breast cancer detection rate based on MDK overexpression was 68% for the metastatic population and 34% for the early-stage population. In the metastatic setting MDK disease detection potential was comparable to that of two conventional markers, carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3) when the three markers were used in isolation (**Table 1**). 92% of the metastatic patients could be detected when all three markers were combined. Interestingly, MDK aberrant expression without the simultaneous overexpression of either of the two conventional markers was observed in ~10% of the metastatic patients. No correlations were observed between MDK levels and specific sites of metastasis, type of treatment, or patient's age at this stage. Although still preliminary, a possible correlation between changes in the MDK levels occurring during the course of treatment and response to treatment as detected through imaging was observed in the metastatic setting.

Table 1: Detection rate of metastatic breast cancer using three tumor markers alone or in different combinations (n=50)

Single Marker/Combination	# of cases detected	Detection rate
CEA	30	60%
CA 15-3	36	72%
MDK	34	68%
CEA/CA 15-3	42	84%
MDK/CEA	43	86%
MDK/CA 15-3	44	88%
MDK/CEA/CA 15-3	46	92%

In the early-stage population MDK was shown to be superior in detecting breast cancer as opposed to the two conventional tumor markers (**Table 2**).

Table 2: Detection rate of early-stage breast cancer using either of three tumor markers (n=50)

Marker	# of cases detected	Detection rate
CEA	8	16%
CA 15-3	6	12%
MDK	17	34%

Discussion

Our preliminary data are supportive of MDK potential as a breast cancer biomarker. Its abnormal elevation in breast cancer patient plasma both in the metastatic and the primary setting (where conventional tumor markers are typically not overexpressed) suggest that MDK can be a valuable tool for detecting/monitoring the disease when conventional markers cannot be used. Our results on MDK applicability as a marker of response to treatment are encouraging although still premature. In the metastatic setting, we observed a pattern in which changes in MDK levels in 3-month follow-up samples compared to baseline could predict the response to treatment as reported through imaging. Additional timepoints and a larger pool of patients are required to solidify our data and reach statistical significance. Taking into consideration that MDK level changes might be preceding changes visible via imaging, assessing imaging data at later time points would be more insightful. Overall, our data are promising but further validation is required for the clinical establishment of MDK.