KALLIKREINS AS MARKERS OF DISSEMINATED TUMOUR CELLS IN OVARIAN CANCER

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ABSTRACT

Background: Kallikreins are a family of 15 recently discovered serine proteases that have been associated with several types of cancer (1,2). The genetic loci of these proteases lie at chromosome 19q13.4. Kallikreins 4, 5, 6, 7, 8, 9, 10, 11, 13, 14 and 15 are elevated in tumour cells, serum or ascites fluid of ovarian cancer patients, at the mRNA and protein levels, and correlate with disease prognosis.

Objective: We hypothesized that kallikreins 4, 5, 6, 7, 8, 9, 10, 11, 13, 14 and 15 could be utilized to monitor dissemination of cancer cells in blood and ascites fluid of ovarian cancer patients.

Methods: We established a sensitive RT-PCR method for detection of KLK6,10 mRNA by utilizing positive or negative kallikrein cell lines. We detected mRNA transcripts in 10 HTB75 cells, an ovarian cancer cell line. We were also able to detect 106 HTB75 cells in a background of 105 kallikrein-negative cells or 100 blood cells. RT-PCR for KLK6 mRNA is disseminated cancer cells from 24 ovarian cancer patients' blood, resulted in 75% positivity, which was not different from the positivity rate of the normal control (80%). Utilizing KLK10 as a marker, we obtained 60% positivity for ovarian cancer patients vs. 20% for controls. Screening of ascitic fluid of 10 ovarian cancer patients revealed 10% positivity for KLK6 and KLK10, compared to 33% for other cancer types. Significant correlations were identified among the mRNA transcripts of kallikreins 4,5,6,7,8,9,10,11,13,14, 15 in cancer cells isolated from ascites fluid of ovarian cancer patients.

Conclusions: We conclude that KLK6 cannot be utilized as a marker for blood dissemination of ovarian cancer cells. KLK transcript numbers from tumor cells that circulate in blood, ascites fluid and lymph nodes may be candidate markers for monitoring metastasis or re-occurrence of ovarian tumor cells in blood (10).

INTRODUCTION

General Information on Human Tissue Kallikreins (hk)

Kallikreins are a family of homologous secreted serine proteases that localize to chromosome 19q13.3-13.4 (1,2).

Certain Kallikreins are candidate biomarkers for the prognosis, detection, and monitoring of ovarian cancer (Table 1)

Several kallikreins are potential biomarkers for breast and prostate cancer (Tables 2 and 3)

HYPOTHESIS

We hypothesized that:

- The number of kallikrein transcripts in tumour cells isolated from blood and tumour cells isolated from ascites fluid of ovarian cancer patients can be utilized as a biomarker for the early diagnosis and prognosis of ovarian cancer.

OBJECTIVES

- Identify a cell line that strongly expresses KLK6 and KLK10
- Determine the sensitivity of the method, using KLK6 and KLK10 as markers
- Check the expression pattern of KLK6 and KLK10 in healthy donor’s blood
- Determine the detection limit of KLK6 and KLK10 in healthy donor’s blood
- Use the method to identify circulating tumor cells in blood of ovarian cancer patients
- Detect KLK 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15 in cancer cells isolated from ascites fluid of ovarian cancer patients.

MATERIALS AND METHODS

We spiked 7 ml of blood with HTB75 cells and we used Dynal beads (C+) to isolate the cells

We isolated tumour cells from ascites fluid of ovarian cancer patients

RESULTS

- HTB75 cDNA mixed with cDNA from 10 LNCaP cells can be detected
- Spiking the blood with HTB75 cells

DETECTION OF TUMOUR CELLS IN ASCITES FLUID (representative results)

We conclude that KLK6 cannot be utilized as a marker for blood dissemination of ovarian cancer cells. KLK transcript numbers from tumor cells that circulate in blood, ascites fluid and lymph nodes may be candidate markers for monitoring metastasis or re-occurrence of the ovarian tumor after surgery

Kallikreins that are coexpressed in ascites fluid may be the subjects of a multiparametric analysis for the detection of ovarian cancer

CONCLUSIONS

- Kallikreins cannot be utilized for monitoring ovarian tumor cell dissemination in blood
- KLK transcript numbers from tumor cells that circulate in blood, ascites fluid and lymph nodes may be candidate markers: for early detection of ovarian cancer - for monitoring metastasis or re-occurrence of the ovarian tumor after surgery
- Kallikreins that are coexpressed in ascites fluid may be the subjects of a multiparametric analysis for the detection of ovarian cancer

REFERENCES


OBJECTIVES

- Check the sensitivity of the method, using KLK6 and KLK10 as markers
- Screen of ascites fluid of 10 ovarian cancer patients revealed 90% positivity for KLK6 and KLK10, compared to 33% for other cancer types. Significant correlations were identified among the mRNA transcripts of kallikreins 4,5,6,7,8,9,10,11,13,14, 15 in cancer cells isolated from ascites fluid of ovarian cancer patients.

We conclude that KLK6 cannot be utilized as a marker for blood dissemination of ovarian cancer cells. KLK transcript numbers from tumor cells that circulate in blood, ascites fluid and lymph nodes may be candidate markers for monitoring metastasis or re-occurrence of the ovarian tumor after surgery

Kallikreins that are coexpressed in ascites fluid may be the subjects of a multiparametric analysis for the detection of ovarian cancer

FUTURE STUDIES

- Examine other Kallikreins as possible biomarkers to detect ovarian tumor cells in blood (9)
- Use a high throughput approach to identify the sensitivity of the method
- Study a large number of patients

STATISTICAL ANALYSIS (asctes fluid)

DETECTION OF TUMOUR CELLS IN ASCITES FLUID

Ovarian cancer patients (n=10)
- 90% of samples were positive for KLK 5, 7, 8 and 9
- 100% of samples were positive for KLK 10, 11 and 13
- 70% of samples were positive for KLK14 and 15
- 60% of samples were positive for KLK4

Gynaecological tumours other than ovarian cancer (n=2)
- 50% of samples were positive for KLK4,5,6,7,8,9,10,11,13
- 8% of samples were positive for KLK14
- 100% of samples were positive for KLK15

Non-gynaecological tumours (n=4)
- 50% of samples were positive for KLK7,9,10,11,13,14,15
- 8% of samples were positive for KLK15

Table 4: Correlations between kallikreins in cells isolated from ascites fluid

Table 1: Ovarian cancer Biomarkers

Table 2: Breast cancer Biomarkers

Table 3: Prostate cancer Biomarkers

Table 4: Detection of Angiotensin Converting Enzyme (ACE) in Tumour Cells