Novel Biomarkers (Kallikreins) for Prognosis and Therapy Response in Ovarian cancer

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EORTC-NCI-ASCO Meeting, November 16, 2007
Within the entire human genome, kallikrein genes represent:

1. the **largest cluster** of contiguous protease genes of any kind
2. the **largest group** of serine proteases

Kallikreins as Cancer Biomarkers
Discovery of a New Biomarker
(*classical method*)

- Discovery of the gene
- **Recombinant protein**
- Antibodies
- **Assays**
- Preliminary Studies
- Detailed Studies
- **Clinical Acceptance**

It took us 7 years to set-up KLK ELISAS

Efforts to improve assays are still ongoing
Kallikrein ELISAs

Developed/Published

- KLK4
- KLK5
- KLK6
- KLK7
- KLK8
- KLK9
- KLK10
- KLK11
- KLK12
- KLK13
- KLK14
- KLK15
Kallikrein 6
A Novel Ovarian Cancer Biomarker
KLK6 in serum

High Serum KLK6 in Ovarian Cancer

- Associated with: (p < 0.001)
  - Late stage
  - High grade
  - Serous histotype
  - No response to chemotherapy
  - Residual tumor
  - Decreased disease-free & overall survival

Independent & Unfavorable Prognostic Indicator

Survival Analysis and Serum KLK6

KLK6 Protein Expression in Ovarian Tissue Extracts

KLK6 and Histological Subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Median KLK6 Concentration (ng/mg)</th>
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<tbody>
<tr>
<td>Serous</td>
<td>4.401</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>1.322</td>
</tr>
<tr>
<td>Mucinous</td>
<td>0.5604</td>
</tr>
<tr>
<td>Clear Cell</td>
<td>1.673</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>4.043</td>
</tr>
<tr>
<td>Non-Epithelial</td>
<td>0.06796</td>
</tr>
</tbody>
</table>
KLK6 and FIGO stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.3158</td>
</tr>
<tr>
<td>II</td>
<td>3.333</td>
</tr>
<tr>
<td>III</td>
<td>3.838</td>
</tr>
<tr>
<td>IV</td>
<td>3.936</td>
</tr>
</tbody>
</table>
KLK6 mRNA Expression in Ovarian Tissues

Complete concordance between KLK6 mRNA and protein expression points to transcriptional regulation.
Kallikreins Overexpressed in Ovarian Cancer

KLK5, KLK6, KLK7, KLK8, KLK10, KLK11 & KLK14

Verified by:

- Tissue, serum & ascites protein levels
- mRNA (quantitative RT-PCR)
- Bioinformatics (EST & SAGE databases)
  - Digital Differential Display
  - In-Silico Northern

Ovarian Cancer-Blinded Study-Pools

marker: KLK 5

premenopausal controls
geometric mean = 0.55 (1.53)

postmenopausal controls
geometric mean = 0.50 (1.44)

all controls
geometric mean = 0.52 (1.48)

1: Ov-Ca-1
z = 5.27

6: Ov-Ben-1
z = -0.16

8: Br-Ca-2
z = 0.66

11: Br-Ben-1
z = -0.07

2: Ov-Ca-2
z = 7.20

3: Ov-Ca-3
z = 1.28

7: Ov-Ben-2
z = -0.52

9: Br-Ca-3
z = -0.39

4: Ov-Ca-4
z = -0.65

5: Endo
z = 0.63

10: Br-Ca-1
z = -0.24

187: Ov-Ca-1
z = 5.27

6: Ov-Ben-1
z = -0.16

8: Br-Ca-2
z = 0.66

11: Br-Ben-1
z = -0.07

2: Ov-Ca-2
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7: Ov-Ben-2
z = -0.52

9: Br-Ca-3
z = -0.39

4: Ov-Ca-4
z = -0.65

5: Endo
z = 0.63

10: Br-Ca-1
z = -0.24
Ovarian cancer

marker: KLK 6

premenopausal controls
geometric mean = 2.80 (1.37)

disease pool
1: Ov-Ca-1
z = 3.83
6: Ov-Ben-1
z = 0.15
8: Br-Ca-2
z = 0.12
11: Br-Ben-1
z = 1.16

premenopausal controls
geometric mean = 3.12 (1.34)

disease pool
2: Ov-Ca-2
z = 5.42
3: Ov-Ca-3
z = 0.88
7: Ov-Ben-2
z = 0.50
9: Br-Ca-3
z = 0.48

all controls
geometric mean = 2.98 (1.35)

disease pool
4: Ov-Ca-4
z = 0.10
5: Endo
z = 1.94
10: Br-Ca-1
z = 0.88
Ovarian cancer

marker: KLK 8

premenopausal controls
geometric mean = 2.14 (1.33)

postmenopausal controls
geometric mean = 2.03 (1.36)

disease pool
1: Ov-Ca-1
z = 1.88
6: Ov-Ben-1
z = -1.74
8: Br-Ca-2
z = -1.27
11: Br-Ben-1
z = 0.15

disease pool
2: Ov-Ca-2
z = 2.41
3: Ov-Ca-3
z = -0.04
7: Ov-Ben-2
z = 0.06
9: Br-Ca-3
z = -0.67

disease pool
4: Ov-Ca-4
z = -0.16
5: Endo
z = 0.42
10: Br-Ca-1
z = 0.14
Ovarian cancer

marker: KLK 10

disease pool
1: Ov-Ca-1  
z = 4.23  
6: Ov-Ben-1  
z = -0.57  
8: Br-Ca-2  
z = -0.97  
11: Br-Ben-1  
z = 0.92

premenopausal controls
geometric mean = 1.41 (1.41)

disease pool
2: Ov-Ca-2  
z = 4.48  
3: Ov-Ca-3  
z = 0.73  
7: Ov-Ben-2  
z = 0.10  
9: Br-Ca-3  
z = 0.22

postmenopausal controls
geometric mean = 1.18 (1.56)

disease pool
4: Ov-Ca-4  
z = -0.42  
5: Endo  
z = -0.16  
10: Br-Ca-1  
z = 0.20

all controls
geometric mean = 1.27 (1.51)
Ovarian cancer

marker: KLK 11

premenopausal controls
geometric mean = 0.43 (2.51)

disease pool
1: Ov-Ca-1
z = 2.37
6: Ov-Ben-1
z = 0.42
8: Br-Ca-2
z = 0.14
11: Br-Ben-1
z = 0.62

postmenopausal controls
geometric mean = 0.48 (2.60)

disease pool
2: Ov-Ca-2
z = 2.61
3: Ov-Ca-3
z = 1.07
7: Ov-Ben-2
z = 0.47
9: Br-Ca-3
z = 0.22

all controls
grothetic mean = 0.46 (2.55)

disease pool
4: Ov-Ca-4
z = 0.56
5: Endo
z = 0.47
10: Br-Ca-1
z = 0.89

frequency
EDRN-Ovarian Study Validation (Serum)

- PLCO samples (approx. 1,000; mix normal/benign/early and late stage ovarian cancer)

- 30-40 biomarkers and proteomic patterns under evaluation

- Blinded

- Identify individual markers and panels

Results will be available early next year
Multiparametric KLK Prognostic Panel

- We measured nine KLKs in ovarian cancer cytosolic extracts
- Developed multiparametric models for prognosis and prediction of patient response to chemotherapy

Multiparametric KLK Prognostic Panel

- Individual Markers-Patient Classification

Multiparametric KLK Prognostic Panel

- Biomarker Combinations-Patient Classification

Multiparametric KLK Prognostic Panel

- Patient Outcome (PFS) at 1 year

Combined marker for 1 year: KLK6, KLK8, KLK11 and KLK13

Multiparametric KLK Prognostic Panel

- **Response to chemotherapy**

Combined Marker
CR/PR : NC/PD

AUC: 0.76 (0.70, 0.85)
95% CI

Combined Marker + Clinical
CR/PR : NC/PD

AUC: 0.90 (0.86, 0.96)
95% CI

Clinic Only 0.74 (0.64, 0.86)

Group of Biomarkers (CA 125, KLK8, KLK13)

KLK Gene Dysregulation in Cancer

- Translocation of KLK locus in cancer
  - Breast cancer cell line: MDA-MB-468
  - FISH analysis

- FISH using the whole chromosome paint 20 (green) co-hybridizes with the 19q13.3/4 BACs (red) confirming previous SKY data suggesting that chromosome 19 material was translocated to chromosome 20.

CONCLUSION: Low copy number gain in this hyper-diploid line (pseudotriploid). Two copies reside at the resident chromosome 19 and the other copy was translocated to chromosome 20.

Bayani et al. Submitted 2007
Unbalanced Translocations of the KLK Locus in Ovarian Cancer

- Very frequent in cell lines (ovarian and breast)
- Translocations found in 8 out of 8 (100%) of ovarian cancer patients
- Relationship of translocations to KLK dysregulation (up or down-regulation) is currently under investigation
- Role of translocations to prognosis is under investigation

Bayani et al. Submitted 2007
KLKs are among the most promising new cancer biomarkers but they need additional validation.

- Diagnostic, prognostic, predictive

- Panels with other biomarkers may be the way to the future

- Understanding their physiology/pathobiology may lead to better clinical applications, including therapeutics