Up-regulation of the potential ovarian cancer biomarker, human kallikrein 6

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ABSTRACT

Introduction: Human tissue kallikreins (hKs) are a family of secreted serine proteases. Twelve hKs, including hK6, are significantly up-regulated in ovarian carcinoma (OvCa). We undertook a pilot study to delineate the mechanism of hK6 up-regulation in OvCa and further examine the value of hK6 as an OvCa biomarker.

Materials & Methods: Cytosolic extracts from ovarian tissues were analyzed with an enzyme-linked immunosorbent assay (ELISA). Results were statistically analyzed to determine the prognostic value of hK6. Selected samples of all four types of specimens were subjected to total RNA and genomic DNA extraction. To determine the relative abundance of the two most dominant KLK6 transcripts, we reverse-transcribed the mRNA and differentially PCR-amplified these two transcripts. Furthermore, for the genomic DNA of each sample extracted, the KLK6 promoter and 6 exons were amplified and sequenced to screen for mutations. To examine the role of DNA methylation and hormonal effects on transcription, normal ovarian and ovarian cancer cell lines were treated separately with the methylation inhibitor 5'-aza-2'-deoxycytidine as well as 5 steroid hormones.

Results: hK6 content (ug/mg of total protein) of malignant tumor tissues was significantly upregulated. Univariate and multivariate Cox regression analyses revealed that patients with hK6-positive tumors had a shorter progression-free survival and overall survival than hK6-negative patients (p<0.005). hK6 up-regulation was concordant at the mRNA and protein levels. Alternative transcript-1 (AT1) was the dominant transcript in all samples studied. Genomic sequencing identified two linked SNPs in the 5'-UTR region of AT1. Neither correlated with hK6 expression. Lastly, neither methylation inhibition nor hormonal treatments produced significant effects on hK6 expression.

Conclusions: We confirm that hK6 is a marker of unfavorable prognosis in OvCa. Its significant overexpression occurs during early stages of the disease and is likely under transcriptional regulation.

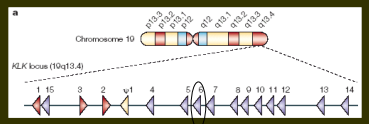
BACKGROUND

Ovarian cancer:

- The most lethal gynecologic malignancy for women in the industrialized countries.
- Independent prognostic biomarkers can facilitate disease management.

Human tissue kallikreins (hKs): Potential prognostic markers

- Secreted serine proteases
- Concurrent upregulation of 12 hKs at in ovarian cancer



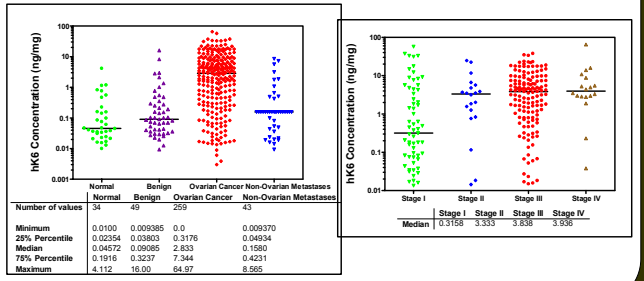
RATIONALE & HYPOTHESIS

- Few studies have examined the processes responsible for the upregulation of the kallikreins in ovarian cancer
- Pilot study on hK6 will shed light on the regulation of the other concurrently upregulated kallikreins
- We hypothesize that hK6 is under transcriptional regulation in ovarian cancer

EXPERIMENTAL DESIGN

1. Quantify hK6 protein expression in ovarian cancer tissues
2. Evaulate prognostic significance of hK6 protein
3. Examine KLK6 mRNA expression in ovarian cancer tissues
4. Examine relative abundance of 2 KLK6 alternative transcripts
5. Conduct genomic DNA mutation screen on KLK6
6. Examine the role of steroid hormones on hK6 expression
7. Examine the role of DNA methylation on hK6 expression

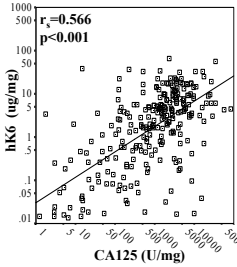
hK6 PROTEIN UPREGULATION IN OVARIAN CANCER

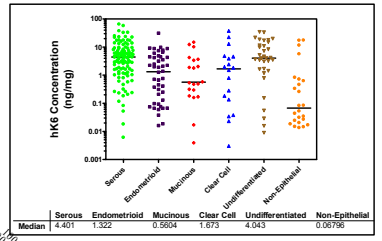


	Normal	Benign	Ovarian Cancer	Non-Ovarian Metastases
Number of values	34	49	259	43
Minimum	0.0100	0.009385	0.0	0.009370
25% Percentile	0.02354	0.03803	0.3176	0.04934
Median	0.04572	0.09085	2.833	0.1580
75% Percentile	0.1916	0.3237	7.344	0.4231
Maximum	4.112	16.00	64.97	8.565

	Stage I	Stage II	Stage III	Stage IV
Median	0.3158	3.333	3.838	3.936

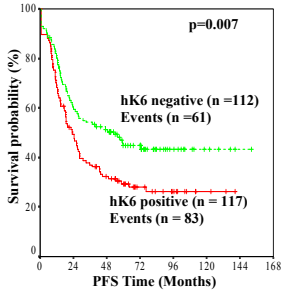
hK6 CORRELATION WITH CA125 AND HISTOTYPE

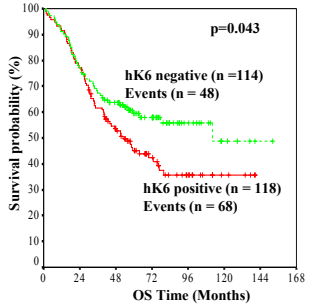




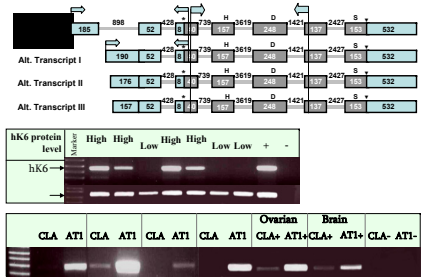
	Serous	Endometrioid	Mucinous	Clear Cell	Undifferentiated	Non-Epithelial
Median	4.401	1.322	0.5604	1.673	4.043	0.06796

PROGNOSTIC SIGNIFICANCE OF hK6

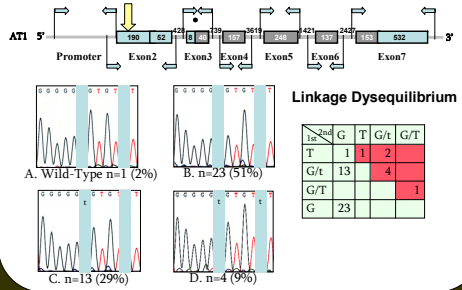




CONCORDANT EXPRESSION OF hK6 mRNA AND PROTEIN



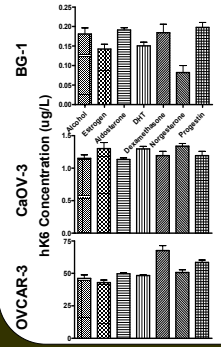
GENOMIC MUTATION SCREENING



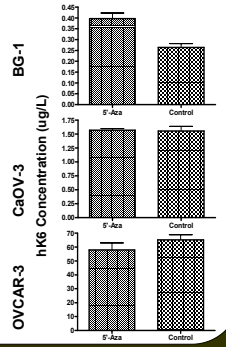
Linkage Disequilibrium

2nd / 1st	G	T	G/t	G/T
T	1	1	2	
G/t	13		4	
G/T				1
G	23			

STEROID HORMONE



DNA METHYLATION



MATERIALS & METHODS

Tissue cytosol from 259 malignant, 49 benign, 43 non-ovarian metastatic tumors, and 34 normal ovarian tissues were extracted and analyzed with an enzyme-linked immunosorbent assay (ELISA) using two hK6-specific monoclonal antibodies. The strength of association between hK6 expression and other clinicopathological variables were statistically determined. The prognostic value of hK6 was evaluated using univariate and multivariate Cox analyses and Kaplan-Meier survival curves.

Selected samples were subjected to total RNA and genomic DNA extraction. To determine the relative abundance of the two most dominant KLK6 transcripts, we reverse-transcribed the mRNA and differentially PCR-amplified these two transcripts. For the genomic DNA of each sample extracted, the KLK6 promoter and 6 exons were amplified and sequenced to screen for mutations.

One normal ovarian and three ovarian cancer cell lines were treated separately with the methylation inhibitor 5'-aza-2'-deoxycytidine as well as 5 steroid hormones: norgestrel, aldosterone, dexamethazone, estradiol, and dihydrotestosterone (DHT) to examine the role of DNA methylation and steroid hormones on hK6 regulation.

CONCLUSIONS

- hK6 is significantly up-regulated in ovarian cancer
- hK6 up-regulation is concordant at mRNA and protein level
- hK6 is under transcriptional regulation
- No differential expression patterns of alternative mRNA transcripts
- No genomic DNA mutations in 7 exons and 5'-flanking region sequenced
- 2 linked SNPs exist in 5'UTR of AT1 but is not related to hK6 expression
- hK6 expression in ovarian cancer is not under the influence of DNA methylation or steroid hormones

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