CAGE法を用いた子宮体癌におけるリンパ節転移予測マーカーの同定

Identification of molecular markers associated with lymph node metastasis in uterine endometrial cancer

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Abstract

Purpose: Lymphadenectomy is recommended in endometrial cancer (EM Ca) treatment; however, it increases the risk of complications such as lymphedema. Given that lymph node metastases (LN+) are rare in low-risk groups, discrimination between LN+/LN− status before lymphadenectomy would be highly valuable, but no effective methods have been developed to date. In this study, we examine the assessment of gene expression profiles in primary tumor as a diagnostic tool minimizing lymphadenectomy.

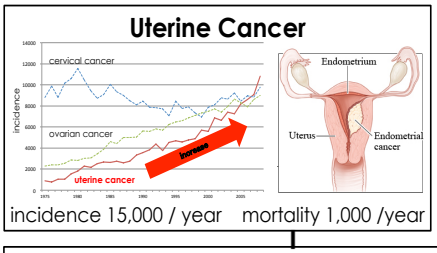
Experimental design: Fifteen EM Ca tissues (5 LN+ and 10 LN–) diagnosed as endometrioid adenocarcinoma Grade 1 with less than one-half muscle invasion were subjected to cap analysis of gene expression (CAGE) and subsequent computational screening. The resulting biomarker candidates were examined by quantitative reverse transcription PCR (qRT-PCR) in 115 EM Ca. In addition, their expression levels were validated using fluorescent in situ hybridization (FISH) and immunostaining.

Results: Genome-wide screening based on CAGE and targeted examination based on qRT-PCR revealed a significant association of *gene X* and *gene Y* isoforms expressed through a novel promoter, with LN+/LN– status in a low-risk group. *Gene X* and *gene Y* isoform were highly expressed in LN– ($p < 0.001$) and LN+ ($p < 0.05$) respectively. The difference in their expression levels was effective in discriminating between LN+/LN- status (AUC = 0.929).

Conclusion: Our study identified two genes, *gene X* and the novel *gene Y* isoform, as biomarkers for assessing LN+/LN– status using EM Ca tissue only. These biomarkers may be used as a diagnostic tool to support clinical decisions that minimize irrelevant lymphadenectomy without increasing recurrence risk.

Background & Purpose

Uterine Cancer



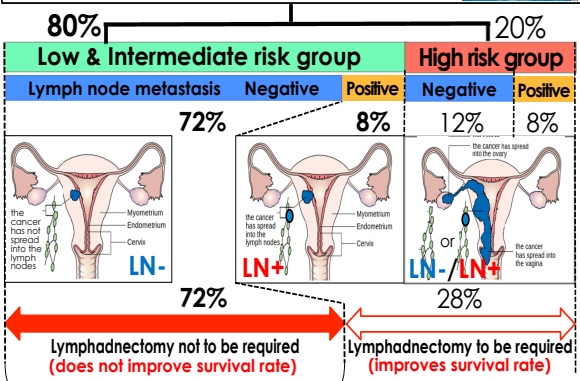
incidence 15,000 / year mortality 1,000 /year

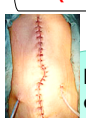
- ✓ Uterine cancer arises from the endometrium.
- ✓ Uterine cancer is the most common gynecological cancer in developed world.

✓ The primary treatment is **surgery**.

hysterectomy + **salpingo-oophorectomy** + **lymphadenectomy**

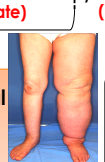
removal of the uterus | removal of both ovaries and tubes | removal of the lymph nodes





Invasion of surgery

- Lengthening the time of a surgery
- more bleeding
- Longer hospital stay
- Larger incision



Physical burden

Lymphedema

- Activity limitation

deterioration of postoperative QOL

- Loss of purpose in life
- Change in her body image

Mental burden

- Diminishing job opportunities
- Risk of unemployment
- Cost burden for medical treatment

Economic burden

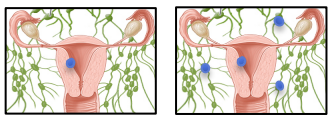
Our purpose

- ✓ **The objective of this study is to establish biomarkers in order to distinguish lymph node metastatic state before surgery.**
- ✓ **It will be realized to improve patient's QOL by avoiding irrelevant lymphadenectomy.**

Method & Results

CAGE is a novel approach for identifying gene expression profiling. CAGE differs from other major gene expression analysis techniques in its capability to accurately identify transcriptional start sites in a genome-wide fashion.

endometrioid adenocarcinoma G1
myometrial invasion ≦ 1/2



LN– LN+

We compared the difference of the gene expressions between Ln– and LN+.

CAGE library preparation

Sequence

Data processing

Quality control
Clustering
Mapping

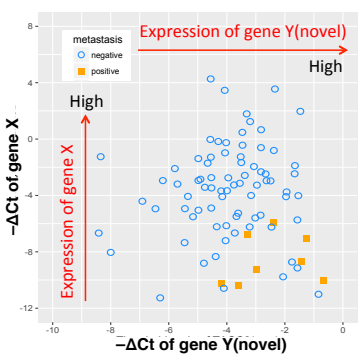
Statistical analysis

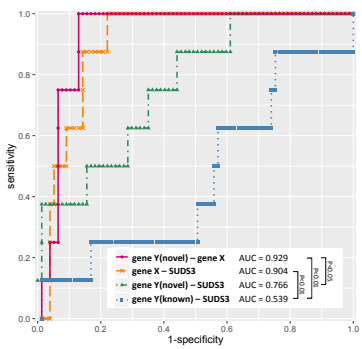
median expression >16 cpm + fold changes of group means >16 + AUC static >95%

Identification of biomarker candidates

validated the candidates by q-RT-PCR

Identification of biomarker for lymph node metastasis





We found that expressions of gene X and novel isoform of gene Y were significantly associated with LN+/– states. The discrimination power of combination marker is further substantial as AUC 0.922. So we believed this combination marker was more effective discrimination of lymph node metastatic states.

Conclusion

- ✓ **Our study identified the two genes, gene X and the novel isoform of gene Y, as potential biomarkers to distinguish LN–/+ state.**
- ✓ **Their combination marker shows more effective discrimination of LN–/+ states.**