

Gene Expression Patterns in a Rat Model of Human Endometrial Adenocarcinoma

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Avhandlingen baseras på följande delarbeten:

- I. Nordlander C, **Karlsson S**, Karlsson A, Sjöling A, Winnes M, Klinga-Levan K, Behboudi A. *Analysis of chromosome 10 aberrations in rat endometrial cancer-evidence for a tumor suppressor locus distal to Tp53*. Int J Cancer. 2007 Apr 1;120(7):1472-81.
- II. **Karlsson S**, Holmberg E, Askerlund A and Klinga-Levan K. *Altered TGF- β pathway expression pattern in rat endometrial cancer*. Cancer Genet Cytogenet. 2007 Aug;177(1):43-50.
- III. **Karlsson S**, Olsson B & Klinga-Levan K. *Gene expression profiling predicts a three-gene expression signature of endometrial adenocarcinoma in a rat model*. Submitted 2008.
- IV. **Karlsson S**, Falck E, Carlsson J, Helenius G, Karlsson M & Klinga-Levan K. *Loss of expression of Glutathione peroxidase 3 in endometrial cancer is correlated with epigenetic mechanisms*. Manuscript.



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Abstract

Endometrial cancer develops from the endometrium of the uterus and is the most common pelvic malignancy diagnosed in women in the western society. Similar to all cancer diseases, endometrial cancer is a disorder that results from complex patterns of genetic and epigenetic alterations involved in the malignant transformation. The BDII/Han rat model is unique for spontaneous hormonal carcinogenesis since more than 90% of the female virgins spontaneously develop endometrial cancer. The possibility to perform global gene expression profiling of tumor cells would likely provide important information of the genes and pathways that are aberrant in endometrial adenocarcinoma (EAC). The works in the present thesis have been focused on investigating the expression patterns in endometrial tumors.

The findings in this thesis involve the identification of a novel candidate tumor suppressor region of rat chromosome 10. This genomic segment contains 18 potential tumor suppressor genes. Preliminary microarray data analysis confirmed that this region might contain relevant candidate genes as the EACs on average had 3.8 times lower expression of *Crk* in comparison to the normal/pre-malignant endometrial tissue cultures. Furthermore, an expression analysis using qPCR, revealed a significant down-regulation of *Myo1c* and *Hic*.

We were also able to identify a group of genes associated with the TGF- β pathway that were differentially expressed between endometrial tumors and normal/pre-malignant endometrium. These results suggest that the TGF- β signaling pathway is disrupted in EAC. This has previously been demonstrated in human EAC, although this is the first report on aberrant expression of TGF- β down-stream target genes.

Evaluation of *Gpx3* down-regulation in the rat EAC cell lines revealed an almost complete loss of expression in a majority of the endometrial tumors. From methylation studies, we could conclude that the loss of expression of *Gpx3* is correlated with biallelic hypermethylation in the *Gpx3* promoter region. This result was confirmed with a demethylation study of EAC cell lines, where the *Gpx3* mRNA expression was restored after treatment with a demethylation agent and a deacetylation inhibitor. We also showed that mRNA expression of the well-known oncogene, *Met*, was slightly higher in endometrial tumors with loss of *Gpx3* expression. A likely consequence of loss of *Gpx3* function is a higher amount of reactive oxygen species (ROS) in the cancer cell environment. Since it has been proposed that overproduction of ROS is required for the hypoxic activation of HIF-1, we suggest that loss of *Gpx3* expression activates transcription of *Met* through induction of the transcription factor HIF-1. The loss of the protective properties of GPX3 most likely makes the endometrial cells more vulnerable to ROS damage and genome instability.

We extended the results obtained from the rat endometrial tumors to human material, and conducted expression analysis of *GPX3* in 30 endometrial human tumors using qPCR. The results showed a uniformly down-regulation of *GPX3* in 29 of the tumors, independent of tumor grade. We thus concluded that the down-regulation of *GPX3* probably occurs at an early stage of EAC and therefore contributes to the EAC carcinogenesis. These results suggest that there are important clinical implications of *GPX3* expression in EAC, both as a biomarker for EAC and as a potential target for therapeutics.

Keywords: rat, complex disease, Endometrial adenocarcinoma, gene expression profiling, cDNA microarrays, candidate genes