**Novel Tumor Suppressor Gene Candidates in Experimental Endometrial Carcinoma**
– From Cytogenetic to Molecular Analysis

Akademisk avhandling

som för avläggande av Filosofi Doktorsexamen vid Institutionen för Cell och Molekylärbiologi, Naturvetenskapliga Fakulteten vid Göteborgs Universitet, kommer att offentligen försvaras i Hörsal Arvid Carlsson, Medicinaregatan 3 torsdagen den 24 september 2009 kl. 9.00

av

**Carola Hedberg**

Fakultetsopponent: Professor James D. Shull, Director of McArdle Laboratory for Cancer Research and Chairman of Department of Oncology, University of Wisconsin-Madison

Avhandlingen baseras på följande arbeten:


UNIVERSITY OF GOTHENBURG
Novel Tumor Suppressor Gene Candidates in Experimental Endometrial Carcinoma
– From Cytogenetic to Molecular Analysis

Carola Hedberg
Department of Cell and Molecular Biology – Genetics
Lundberg Institute, Faculty of Science, University of Gothenburg

Abstract
Endometrial carcinoma (EC) is the most common form of gynecological malignancy, ranking fourth in incidence among tumors diagnosed in women. As is the case with other complex diseases, detailed analyses of the underlying mechanisms of cancer are difficult, due mainly to the genetic heterogeneity of the human population and differences in the environment and lifestyle of individuals. In this sense, analysis in animal models may serve as a valuable complement. The inbred BDII rat strain is genetically prone to spontaneous hormone-related EC and it has been used as a powerful model to investigate molecular alterations in this tumor type. BDII female rats were crossed with males from two non-susceptible rat strains and tumors developed in a significant fraction of the progeny. We subjected a subset of BDII rat tumors to detailed analysis based on the molecular data used for the classification of human ECs. Our analysis revealed that this tumor model can be related to higher grade human type I ECs, i.e. a subgroup of ECs that constitutes more than 80% of this tumor type in humans.

Earlier work using comparative genome hybridization (CGH) revealed that rat chromosome 10 (RNO10) was frequently involved in cytogenetic aberrations in BDII rat tumors. To identify the potential target region(s)/gene(s) for these changes, we subjected a panel of rat ECs to allelic imbalance (AI) analysis. Four distinct regions of recurrent AI were identified. By deriving evolutionary tree models based on AI data, we demonstrated that one of these AI regions (located adjacent to Tp53) was close to the root in the derived onco-tree models, indicating that this segment might harbor early important events. In combined FISH, chromosome paint, gene expression and gene sequencing analyses, we found that, instead of Tp53, the main selection target was a region close and distal to Tp53. We developed a detailed deletion map of this area and substantially narrowed down the size of the candidate region. We then subjected all 19 genes located within this segment to qPCR analysis, followed by statistical analysis of the results, and thus identified the Hic1, Skip and Myo1c genes as potential target(s). By subjecting these genes to DNA sequencing, analysis of protein expression and of epigenetic silencing, we ruled out Hic1 and confirmed Skip and Myo1c as the candidates. Interestingly, it appears that Skip and Myo1c perform overlapping roles in PI3-kinase/Akt signaling, which is known to have implications for the survival and growth of cancer cells. In conclusion, starting from cytogenetic findings and applying a candidate gene approach, we introduced two attractive candidate genes within the independent region of tumor suppressor activity distal to Tp53.

Key words: cancer, endometrial cancer, rat models, rat chromosome 10, deletion, allelic imbalance, FISH, gene expression, tumor suppressor gene, Hic1, Skip, Myo1c

Göteborg 2009