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

Akademisk avhandling

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av

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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:

I.   Samuelson E, **Hedberg C**, Nilsson S and Behboudi A. Molecular classification of spontaneous endometrial adenocarcinomas in BDII rats. *Endocrine-Related Cancer*: *2009; 16: 99-111*.

II.   **Nordlander C**, Behboudi A, Levan G and Klinga-Levan K. (2005) Allelic imbalance on chromosome 10 in rat endometrial adenocarcinomas. *Cancer Genetics and Cytogenetics*: *2005; 156: 158-166*.

III.   Chen L, **Nordlander C**, Behboudi A, Olsson B and Klinga-Levan K. Deriving evolutionary tree models of the oncogenesis of endometrial adenocarcinomas. *International Journal of Cancer*: *2006; 120: 292-296*.

IV.   **Nordlander C**, Karlsson S, Karlsson Å, Sjöling Å, Winnes M, Klinga-Levan K and Behboudi A. Analysis of chromosome 10 aberrations in rat endometrial cancer – evidence for a tumor suppressor locus distal to *Tp53*. *International Journal of Cancer*: *2007; 120: 1472-1481*.

V.   **Hedberg C**, Garcia D, Linder A, Samuelson E, Ejeskär K, Abel F, Karlsson S, Nilsson S and Behboudi A. Characterization of the independent tumor suppressor loci distal to *Tp53* – a candidate gene approach. *Manuscript*.

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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 PI 3-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*.