Neuron Navigator 3 (NAV3) Gene Aberrations in Human Cancer: Copy Number Changes and Target Genes

Emilia Carlsson
Department of Dermatology and Allergology, Institute of Clinical Medicine, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland. E-mail: emilia.carlsson@helsinki.fi

Emilia Carlsson defended her PhD thesis in Helsinki, Finland, on 30th November 2012. The thesis was supervised by Professor Annamari Ranki and PhD Kirsi Niiranen from the Department of Dermatology and Allergology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland. The opponent was Professor Veli-Pekka Lehto, Department of Pathology, Haartman Institute, University of Helsinki, Finland. This thesis can be found at: http://urn.fi/URN:ISBN:978-952-10-8415-4.

This thesis is based on the original observation of an allelic deletion of the recently described *Neuron navigator 3 (NAV3)* gene in patients with primary cutaneous T-cell lymphoma (CTCL) and CTCL-associated lung cancer. Thereafter mutations or copy number changes of *NAV3* have been reported in melanoma, glioblastoma (GBM) and adrenal carcinoma. The aim of this study has been to shed light on the function and interactions of the NAV3 protein, as well as characterizing *NAV3* copy number changes and their effect on patient survival in several forms of cancer.

NAV3 is a novel cancer-associated gene at 12q21. The specific function of *NAV3* is not known except that it carries actin-binding domains with ATPase activity, and is therefore likely to have an action on microtubules and cytoskeleton reorganization. The three Navigator genes, *NAV1*, *NAV2*, and *NAV3* share homology among themselves and among different species. This suggests a central role for the encoded proteins in cell biology.

In this study, *NAV3* copy number changes have been studied by fluorescence *in situ* hybridization (FISH) and array comparative genomic hybridization (aCGH) in non-melanoma skin cancers, colorectal cancer and neural system tumours, and the relevant *NAV3*-regulated target genes have been identified. Furthermore, the expression levels on NAV3 and *NAV3*-regulated signaling molecules have been correlated to disease progression and patient outcome.

In basal cell carcinoma (BCC) and squamous cell carcinomas (SCC) we found *NAV3* copy number loss and corresponding absence of protein in 21% of the BCC and in 25% of the SCC tumours. In the nodular/superficial BCC subgroup, also low-level *NAV3* amplification was found. *NAV3* aberrations were independent of the known chromosome 6 amplification in BCC. Chromosome 12 polysomy, also independent of chromosome 6 polysomy, was found in 33% and 25% the invasive type of BCC and in SCC, respectively.

Emilia Carlsson defended her PhD thesis on *NAV3* gene aberrations in Helsinki in November 2012. Professor Veli-Pekka Lehto was the opponent (*right*) and Professor Annamari Ranki acted as custos and supervisor (*middle*).

In colorectal carcinomas *NAV3* deletion and chromosome 12 polysomy were detected in 30% and 70% of microsatellite-stable (MSS) carcinomas, and in 12.5% and 50% of carcinomas with microsatellite instability, but also in 23% and 30% of adenomas, respectively. Low copy number amplification of *NAV3* was found in 25% of MSS samples.

One hundred and nineteen patient samples representing different central and peripheral nervous system tumours were studied for *NAV3* copy number changes. Neuronally differentiated tumours (neuroblastomas and medulloblastomas) entailed more *NAV3* aberrations than the glial tumours. In glial tumours, those with grade IV (glioblastoma (GBM)) had significantly more *NAV3* deletions than tumours with grades I, II or III. Log rank analysis also linked *NAV3* deletion to a poor prognosis in gliomas. In contrast, glioma patients with *NAV3* amplification showed better prognosis than those with normal *NAV3* copy numbers. The FISH result was also supported by aCGH analysis, which showed results matching the FISH analysis for tumour samples with *NAV3* amplification and deletion.

To understand the *in vivo* functional consequences of *NAV3* copy number changes, especially of *NAV3* deletion, we silenced *NAV3* in normal colon, GBM and primary keratinocyte cells, using a commercially available small inhibitory ribonucleid acid (siRNA) construct. Post transfection RNA samples from several time points were analyzed with Agilent 44K microarray. In GBM and colorectal cell lines we identified, among others, GnRHR and IL-23R as upregulated by *NAV3* gene silencing. The upregulation of the selected genes were confirmed by quantitative PCR. In primary keratinocyte, *NAV3* silencing led to consistent upregulation of 22 annotated genes, and several Wnt/HH pathway genes were slightly overexpressed too.

Taken together the results of this thesis support the previously suggested role of *NAV3* as a novel cancer associated gene, and we suggest that *NAV3* affects the malignant potential of a given tumour through multiple pathways. This assumption is based on the fact that gene expression analysis of NAV3 silenced cells indicates that *NAV3* affects genes with involvement in both inflammation and carcinogenesis.