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.

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 (neuroblastosmas 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 ribonucleic 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.