From 1p3 to PI3K
Studies of Neuroblastoma

AKADEMISK AVHANDLING

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av

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Avhandlingen baseras på följande arbeten:


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Neuroblastoma (NB) is a tumor of the sympathetic nervous system and is the most common extra-cranial tumor of childhood, accounting for 7% of all pediatric malignancies. Despite recent advances in therapeutics, outcome is still fatal for patients with aggressive NB and side-effects of treatment are severe. These are important reasons to gain further knowledge of the biology behind NB.

Aims: The objective of this thesis was to explore genes and gene products that might contribute to initiation and progression of NB and possibly also other malignancies. Main focus has been on the chromosomal region 1p36.2-3 and participants of the PI3K/Akt signaling pathway.

Results: Real-time expression analysis of 30 genes at 1p36.2-3 showed that TNFRSF9 and PIK3CD were down regulated in 1p-deleted compared to non-deleted NB tumors. Studies of the same region showed four genes (ERRFI, CASZ1, RBP7 and PIK3CD) possibly regulated by epigenetically means. Bisulphite sequencing of these four genes in NB cell lines and primary tumors showed that methylation probably is not involved but that histone deacetylation could be implicated in their regulation. Some rare sequence variants were also identified in ERRFI and PIK3CD. PIK3CD encodes a catalytic subunit of the phosphatidylinositol 3-kinase (PI3K) that is involved in activation of Akt. Analysis of mRNA levels in a set of 88 genes associated to PI3K/Akt signaling showed that PDGFRA, PIK3R1, PIK3CD, PRKCBI, PRKCB, PRKCDZ and EIF4EBP1 were differentially expressed comparing stage 1-2 to stage 4 NB. At the protein level a stage-dependent expression of the different catalytic isoforms were detected, where levels of p110δ were higher in stage 4 tumors compared to stage 1-2, while the opposite was seen for p110δ. Stage 4 NB also had higher levels of phosphorylated Akt (T308 and S473) compared to low stage NB. Furthermore, levels of phosphorylated Akt T308 showed inverse correlation to protein levels of Pten.

We have also identified a novel splice variant p37δ, encoded by PIK3CD. Usage of an alternative donor site leads to truncation in the RAS-binding domain and loss of the catalytic domain. Despite the truncation, p37δ interacts with RAS and there is a strong correlation between protein levels of p37δ and RAS in primary cells. Expression of p37δ is increased in human cancers of the ovaries and colon and ubiquitous expression of the human p37δ in Drosophila increased the body size of the fly. Furthermore, over-expression of p37δ in HEK-293 and mouse embryonic fibroblasts increased proliferation and invasive properties compared to controls, indicating a role in tumorigenicity.

Conclusion: Analysis of expression levels of genes and proteins could be used for pinpointing important genes and pathways. This thesis has added more knowledge about the genes at 1p36.2-3, a region commonly deleted in NB, as well as the PI3K/Akt signaling in NB. We have also described a new splice variant of p110δ that is expressed in human cancer and increases proliferation in vitro and in vivo.

Keywords: cancer, tumor, neural crest, mucosa, premalignant, neuroblastoma, tumor suppressor gene, oncogene, gene expression, epigenetics, splicing, signaling, 1p36, PI3K, Akt, Western blot, TaqMan, PIK3CD, PIK3R1, p110δ, p37δ, p85, RAS

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