



**Karolinska
Institutet**

Institutionen för Biovetenskaper och Näringslära

Hedgehog Signaling in Rhabdomyosarcoma: Role of GLI Factors and Splice Variants in Signal Transduction

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
Institutet offentligen försvaras i Hörsalen plan 4, Novum, Huddinge

Torsdagen den 27 januari, 2011, kl 09.30

av

Ulrica Tostar

Huvudhandledare:

Professor Peter G. Zaphiropoulos
Karolinska Institutet
Institutionen för Biovetenskaper och
Näringslära

Bihandledare:

Professor Rune Toftgård
Dr. Takashi Shimokawa
Karolinska Institutet
Institutionen för Biovetenskaper och
Näringslära

Fakultetsopponent:

Professor Fritz Aberger
University of Salzburg
Department of Molecular Biology
Austria

Betygsnämnd:

Docent Theocharis Panaretakis
Karolinska Institutet
Institutionen för onkologi och patologi

Docent Lars-Arne Haldosén
Karolinska Institutet
Institutionen för Biovetenskaper och
Näringslära

Docent Carina Hellberg
Ludwig Institute for Cancer Research
Uppsala

Stockholm 2011

ABSTRACT

Rhabdomyosarcoma (RMS) is the most common soft-tissue childhood cancer. Deregulation of the Hedgehog (HH) signaling pathway, which is essential for proper embryonic development, has been implicated as a driving force for this cancer. In paper I, we have shown that sporadic human RMS have an overactive HH signaling pathway, and exhibit loss of heterozygosity of the two tumor suppressor genes, *PTCH1* and/or *SUFU*, indicating a role for the promotion of rhabdomyoblastic tumor development. Moreover, we also identified a novel *PTCH1* germ-line mutation in a patient suffering from the Nevoid basal cell carcinoma syndrome and also demonstrated that fetal rhabdomyoma (RM), a benign rhabdomyoblastic tumor, is a true component of this disorder. We analysed 12 RM/RMS tumors and 5 E-RMS cell lines for the presence of mutations in *PTCH1*, but none were detected. To evaluate the functional importance of the deregulated HH pathway in specifically in embryonal RMS (E-RMS), we analysed the E-RMS cell lines for their dependence on HH activity (Paper II). All cell lines expressed HH signaling components and displayed upregulated HH target gene expression. Inhibition of HH signaling activity by the use of two small molecule antagonists, cyclopamine and GANT61, led to reduced proliferation of the cell lines. The effect of GANT61 was specific as HH target gene expression was reduced, whereas cyclopamine gave off-target effects. GANT61 induced apoptosis, and significantly reduced tumor growth in an *in vivo* model. Knockdown of the GLI transcription factors, the ultimate effectors of HH signaling, revealed that GLI1 and GLI3 were important for cell proliferation, whereas GLI2 was dispensable. As GANT61 inhibits GLI1/GLI2 transcriptional activity, the inhibition of E-RMS growth is likely to be mediated through GLI1.

The HH pathway is a very complex and highly regulated pathway, and the complexity is further increased by the presence of several isoforms of HH pathway components. In paper III, we identified and analysed a novel GLI1 splice variant, which is generated by skipping exons 2 and 3 and encodes an N-terminal truncated GLI1 protein (GLI1ΔN). The expression of this variant is downregulated in tumor tissues compared to normal samples. GLI1ΔN was upregulated by HH signaling to the same extent as full-length GLI1, but generally had a weaker capacity to activate transcription.

Another negative regulator of HH signaling, SUFU, has also several isoforms. In paper IV, we have analysed a C-terminal truncated variant, SUFU-ΔC, for its impact on HH signal transduction. SUFU-ΔC mRNA was expressed at similar levels as SUFU-FL, but on the protein level only very low amounts of SUFU-ΔC could be detected in E-RMS cell lines. Although SUFU-ΔC was shown to be less stable than SUFU-FL, it possesses an equal ability to repress GLI2 and GLI1ΔN, but not GLI1FL transcriptional activity. Co-transfection of SUFU-ΔC and SUFU-FL resulted in increased protein expression levels relative to individual transfections, implying a protein stabilizing capacity of the SUFU variants.

In conclusion, we have shown a major role for the HH signaling pathway in the establishment and maintenance of RMS tumors. The analyses of the GLI1 and SUFU splice variants reveal increased complexity, and suggest novel regulatory mechanisms in the HH signaling pathway, in RMS but also in other HH pathway-related tumors.