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Analysis of novel Wnt pathway modulators and genetic aberrations in colorectal cancer by functional genomics, biochemical analysis and deep sequencing

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Cellular signaling pathways represent promising targets for novel, selective therapeutic strategies. To understand the interplay between different factors of signaling cascades involved in the carcinogenic process, detailed studies of signaling pathways and their components are needed. Moreover, to screen for predictive mutations or targetable alterations in clinical studies and practice, sophisticated methods such as deep-sequencing need to be implemented. This thesis therefore focuses on two aspects of molecular medicine: On the identification and functional analysis of a novel regulator of the Wnt signaling pathway and on the establishment and assessment of targeted deep sequencing for archived colorectal cancer (CRC) samples.

In the first part of the thesis, structural genomic data from patients with CRC were combined with a functional genome-wide RNAi screen for novel regulators of the Wnt pathway, a signaling cascade known to be activated in more than 90% of CRCs. This approach identified the SSXT-domain containing protein SS18-like 1 (SS18L1/CREST). Silencing SS18L1 in CRC cells led to decreased Wnt signaling activity and markedly reduced proliferation of CRC cells. SS18L1 interacted with the nuclear β-catenin-TCF4 complex, which is central for transcriptional regulation of Wnt target genes. Domain-deletion mapping showed that the N-terminal region of β-catenin was necessary for interaction with SS18L1. Marked copy number gains and overexpression of SS18L1 were observed in CRC.

In the second part, amplicon sequencing of formalin-fixed paraffin-embedded (FFPE) and paired frozen samples from CRC metastases was established and comparatively evaluated with three different bioinformatic analysis pipelines. 212 amplicon regions in 48 cancer related genes were sequenced with Illumina MiSeq. In resection specimen from 16 patients with CRC liver metastases, 29 non-synonymous coding mutations were identified in eleven genes. Most frequent were mutations in TP53 (10), APC (7), PIK3CA (3) and KRAS (2). Sample quality of FFPE tissues, determined by the amount of amplifiable DNA in a qPCR assay, was poor in almost half of the samples. Nevertheless, a high concordance of mutations detected in FFPE and paired frozen tissue was observed in ten matched samples with an adapted Unified Genotyper analysis pipeline, revealing 21 identical mutation calls and only two mutations differing. Comparison of these results with two other commonly used variant calling tools, however, showed high discrepancies.

Taken together, in this thesis, SS18L1 was identified and characterized as novel regulator of Wnt signaling. The increased expression of SS18L1 observed in a subset of CRC patients could enhance Wnt signaling and disease development. Amplicon sequencing prove to be a suitable tool for molecular characterization of hot spot mutations in clinical CRC samples. However, remarkable differences observed among results of different variant calling tools highlight the need for standardization and benchmarking, which will be required for translational and clinical applications.