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Dissertations-Kurzfassung

Validation and Characterization of MEK inhibitor induced Wnt signaling in Colorectal Cancer Cell Lines

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Increased Wnt and MAPK signaling activity due to respective mutations are hallmarks of colorectal cancerogenesis. Therefore, pharmacological perturbation of both signaling pathways was hoped to yield therapeutic potential for treatment of advanced colorectal carcinoma. Whereas treatment with therapeutic antibodies directed against epidermal growth factor receptors significantly improves survival of patients with RAS wild type tumors, patients whose tumor express RAS mutations do not profit from a respective therapy and have very limited therapeutic options. Disappointingly, MEK1/2 inhibitors that target the MAPK pathway downstream of RAS and RAF did not significantly improve survival of colorectal cancer patients with BRAF or RAS mutant tumors in clinical trials when applied as monotherapy or in combination with other targeted agents despite promising preclinical data.

Compound screens previously performed in the Boutros group in search of novel Wnt pathway modulators identified MEK1/2 inhibitors as activators of Wnt signaling in KRAS mutant Wnt reporter expressing colorectal cancer cell lines. The aims of this thesis were to validate this finding with additional methodological approaches and to identify underlying mechanisms.

To this end, Wnt target gene AXIN2 was determined in MEK1/2 inhibitor treated colorectal cancer cell lines with differential mutational backgrounds, and the role of individual MAPK and Wnt signaling pathway members was determined by RNA interference and pharmacological perturbation. Moreover, a multiplexed Western blot (DigiWest) was performed to identify members of both pathways abundantly regulated upon MEK inhibitor treatment. Candidate proteins were subsequently evaluated with respect of a functional role in mediating MEK induced Wnt signaling.

The data presented in this thesis confirmed the property of MEK inhibitors to activate canonical Wnt signaling on target gene level in a subset CRC cell lines. A role of MEK1/2 and downstream kinases ERK1/2 in mediating Wnt signaling was underscored by small-interfering RNA knockdown experiments that confirmed Wnt pathway activation upon perturbation of MEK and ERK and thereby excluded a relevant role of drug off-target effects. In search of mechanisms underlying the Wnt activation by MEK inhibitors, a relevant role of β-Catenin was identified as RNA interference and pharmacological perturbation of CTNNB1 abrogated the Wnt activating effect. Additionally, reduction of Axin1 protein levels upon MEK inhibition was identified by DigiWest (a novel multiplexed Western blot technology) in HCT116 cells and confirmed by conventional Western blot experiments. Due to its key role as a negative Wnt regulator and member of the Wnt destruction complex, the role of Axin1 in mediating Wnt activation was further investigated. Follow-up experiments performed by colleagues supported a functional role of Axin1 reduction in mediating MEK1/2 inhibitor induced Wnt signaling in HCT116 cells and characterized the underlying mechanism.

The data presented in this thesis and obtained from subsequent experiments performed in the Boutros group highlight the complexity of crosstalks between MAPK and Wnt signaling and the potential negative implications of inadvertent Wnt signaling induction. Taken together, they provide a rationale for further pre-clinical characterization of combinatorial treatment strategies that simultaneously target Wnt and MAPK signaling pathways in models of colorectal cancer. This may finally result in clinical testing of novel treatment strategies for advanced CRC combining MEK and Wnt pathway inhibition.