Systematic identification of druggable PKA substrates involved in colon cancer progression

SpT-26-5

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Deregulation of G protein coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. Examples are activating mutations in the AC-stimulatory Gαs proteins (GNAS), which occur in 4,2% of all tumors. These lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA) pathway. In order to identify druggable PKA-effector proteins, we determined the phospho-proteomic composition of macromolecular PKA complexes from a collection of Gαs-mutated cancer cells and human glioblastoma biopsies. Using a substractive phospho-proteomic approach, we identified a multitude of proliferation-relevant PKA substrates and selected two druggable and cancer-implicated candidates for closer examination, namely the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and Tripartite motif 28 (TRIM28) respectively. PFKFB3 is a key modulator of glycolysis, implicated in maintaining cancer cell metabolism. We showed that nuclear PFKFB3 acts as a PKA substrate. Moreover, small molecule mediated inhibition of PFKFB3 reduced proliferation of Gαs-mutated colon cancer cells. Further, besides quantitative metabolite analyses of the cellular glycolytic flux, we revealed a nuclear function of PFKFB3. Using the RNAseq technology TUCseq we recorded immediate transcriptome changes upon PFKFB3 inhibition. Thus, we gained evidence for a possible link of PFKFB3 to p53 function in the studied colon cancer cell setting. TRIM28, the second novel PKA substrate, supports tumor progession through ubiquitination of the tumor suppressor p53. We investigated changes in protein stability of known anti-oncogenic TRIM28 ubiquitination substrates upon kinase activation. Currently, we explore a polypharmacology approach by inhibiting nuclear TRIM28 and PFKFB3 functions which may hamper proliferation of selected colon cancer cells.

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