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A protein-interface disrupting 23-mer peptide is a prototypic H-Ras-nanocluster and -signalling disruptor

The three RAS genes, *HRAS, NRAS* and *KRAS*, are mutated in 19 % of cancers and individual rare, but collectively common developmental diseases called RASopathies. Despite the recently approved KRAS-G12C inhibitors Sotorasib and Adagrasib, there are only very few other treatment options for *RAS* mutant diseases.

Here we present a novel, prototypic inhibition strategy, which aims at disrupting the nanoscale signaling hubs of H-Ras on the plasma membrane, called nanocluster.

In our previous model we proposed, that the dimeric nanocluster scaffold galectin1 (Gal1) interacts with the Ras binding domain (RBD) of the Ras-effector B-Raf. It thus stabilizes a complex of stacked dimers of Ras and Raf, which may represent the minimal unit of active nanoclusters. Given that nanoclustering determines MAPK signal output, we hypothesized that interference with the Gal1/ Raf-RBD interaction represents an innovative opportunity to normalize Gal1 augmented Ras-MAPK signaling.

We first provide further supportive evidence for our model and establish that the B-Raf preference emerges from differences between the RBDs of Raf paralogs. Then we elaborate that the here identified L5UR peptide binds with low micromolar affinity to the B-Raf-RBD. Importantly, the 23-mer core fragment of the L5UR peptide is sufficient to disrupt the Gal1/ B-Raf-RBD interaction and thus Gal1-enhanced H-Ras nanocluster. This activity is sufficient to reduce MAPK-output and cell viability in *HRAS*-mutant cancer cell lines.

Our data suggests that Raf proteins are integral components of active Ras nanoclusters and that the interface of Gal1 and the Raf-RBD can be targeted to disrupt Gal1 enhanced H-Ras nanocluster.