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GPR133 (ADGRD1) signaling is increased by the dissociation of its extracellular NTF and by binding of its new positive allosteric modulator PTK7.

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The adhesion G protein-coupled receptor GPR133 (ADGRD1), supports growth of the brain malignancy glioblastoma (GBM), and signals through $G_{\alpha s}$-mediated cAMP increases. We recently demonstrated in patient-derived GBM cultures and HEK293T cells that GPR133 is intramolecularly cleaved into N-terminal and C-terminal fragments (NTF and CTF) early in the endoplasmic reticulum, and that these fragments remain non-covalently bound as hetero-dimers until reaching the plasma membrane. At the plasma membrane, we observed dissociation of the extracellular NTF from the transmembrane-spanning CTF, which acutely increases receptor signaling. While this recent study shed light on receptor processing and the dissociation-mediated mechanism of activation, the extracellular interactome of GPR133 in GBM and its influence on NTF shedding and GPR133 activation, remained unknown. To answer these questions, we used an affinity copurification and mass spectrometry approach in patient-derived GBM cells and identified the membrane protein PTK7 as an extracellular binding partner of GPR133. PTK7 binds the autoproteolytically generated NTF of GPR133 and its expression on neighboring cells *in trans* increases GPR133 signaling. In agreement with an NTF-dissociation mediated mechanism, this agonistic effect requires the intramolecular cleavage of GPR133 as well as PTK7's tethering to the plasma membrane or rigid extracellular substrates. Interestingly, PTK7 binding *in trans* also facilitates activation of GPR133 by soluble peptides mimicking the endogenous tethered *Stachel* agonist, suggesting that PTK7 binding allosterically enhances accessibility of GPR133's orthosteric *Stachel* binding pocket. Overall, these findings suggest that PTK7 acts as a positive allosteric modulator of GPR133 signaling *in trans* via a mechanism dependent on both cleavage of GPR133 and plasma membrane insertion of PTK7.

GPR133 and PTK7 are expressed in adjacent cells in glioblastoma, where knockdown of either protein results in the tumor cells' loss of viability, as well as several non-pathological tissues in the human body, which suggests that this novel ligand-receptor interaction may be relevant in both health and disease.