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"Hot spots" for pathogenic gain-of-function mutations in transmembrane domains of receptor tyrosine kinases

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Motivation and Aim: Signal transduction by receptor tyrosine kinases (RTKs) and related receptors has been in the spotlight of scientific interest owing to the central role of these single-spanning membrane proteins in the regulation of development, cell motility, proliferation, differentiation, and apoptosis. Nowadays, the elucidation of high-resolution structure of full-size RTK having flexible multiple-domain composition is still a challenge. During signal transduction across plasma membrane, RTKs are activated by proper ligand-induced homo- and hetero-dimerization or by reorientation of monomers in preformed receptor dimers upon ligand binding. Specific helix-helix interactions of transmembrane domains (TMD) are believed to be important for RTK lateral dimerization and signal transduction. Either destroying or enhancing such helix-helix interactions, e.g. by mutagenesis, can result in many human diseases: developmental, oncogenic, neurodegenerative, immune, cardiovascular etc.
Methods and Algorithms: We experimentally determined the alternative dimeric conformations of the TMDs of several representatives of RTK and related receptors in different membrane-mimicking environments using integrative structural biology methods, including high-resolution NMR spectroscopy combined with MD-relaxation in explicit lipid bilayer. Based on the available mutagenesis and clinical data about localization of gain-of-function mutation, observed conformations correspond to the dormant and active states of these receptors.
Results: Fine adaptation of intermolecular polar and hydrophobic contacts that we found to accompany the different dimerizations of human epidermal growth factor receptor (HER) TMDs (observed in detergent micelles or in lipid bicelles) suggests that certain membrane properties can govern the TMD helix-helix packing and, thus, their alteration can trigger the receptor state. Whereas two distinct dimeric modes of growth hormone receptor (GHR) TMD (coexisting in micellar environment) revealed the functional role of juxtamembrane region rearrangements in alternation between protein-protein and protein-lipid interactions that can be initiated by ligand binding. Pathogenic transmembrane mutations found for the HER relatives are located as a rule in narrow regions within the specific TMD helix-helix interfaces assuming that the intermolecular interactions inside membrane are important for the RTK cell signaling dysfunction in human organism [1, 3]. Such regions can be characterized as a "hot spot" for gain-of-function mutations associated with different human pathologies.

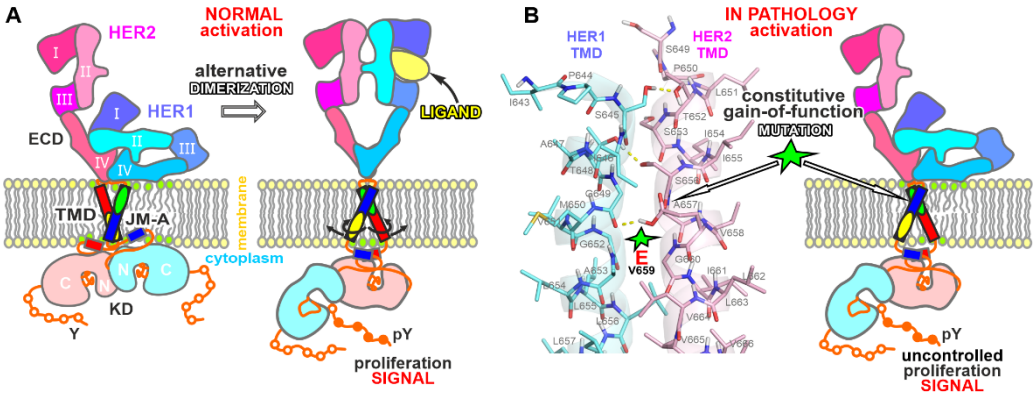

Fig. 1. Normal and pathological activation of HER1/HER2 hetero-dimer as a model RTK. (A) Presumable structural rearrangements of HER1/HER2 hetero-dimer in the course of ligand-induced receptor activation according to the lipid-mediated rotation-coupled mechanism proposed in [1]. (B) Additional inter-monomeric hydrogen bonding caused by HER transmembrane polar mutations (e.g. V659E-HER2) can stabilize the N-terminal dimerization mode of TMD allowing release of cytoplasmic juxtamembrane regions (JM-A) from the membrane leaflet and activation of kinase domains (KD) independently of ligand binding and regardless of the states of extracellular domains (ECD) and surrounding lipids [3]

Conclusion: Observed the RTK TMD helix-helix packing diversity appears in favor of the recently proposed the lipid-mediated rotation-coupled activation mechanism [1], which implies that the sequence of structural rearrangements of RTK domains is associated with perturbations of the lipid bilayer in the course of ligand-induced receptor activation, considering the receptor together with its lipid environment as a self-consistent signal transduction system (Fig. 1A). The finding of the pathogenic mutation "hot spots" justifies a prediction that similar gain-of-function mutations, e.g. enhancing the TMD dimerization in certain conformation and thus activating the receptor independently of ligand binding (Fig. 1B), can be found for other RTK representatives and suggests searching for them is a promising idea for future clinical studies. It can also have potential therapeutic implications, broadening the spectrum of targets for pharmaceuticals by inclusion of plasma membranes and their constituents.

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References

1. Bocharov E.V. et al. Helix-helix interactions in membrane domains of bitopic proteins: Specificity and role of lipid environment. *Biochim Biophys Acta Biomembr.* 2017;1859(4):561-576.
2. Ou S.I., Schrock A.B., Bocharov E.V. et al. HER2 transmembrane domain (TMD) mutations (V659/G660) that stabilize homo- and heterodimerization are rare oncogenic drivers in lung adenocarcinoma that respond to afatinib. *J Thorac Oncol.* 2017;12(3):446-457.
3. Bocharov E.V. et al. Structural basis of the signal transduction via transmembrane domain of the human growth hormone receptor. *Biochim Biophys Acta Gen Sub.* 2018;1862(6):1410-1420.