Characterization of novel partners of the RHOB GTPase in response to EGFR inhibitors

Lung cancer remains the leading cause of cancer death worldwide. Approximately 15-20 % of these cancers carry an activating mutation in the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK) essential for cell survival. As these tumors do not respond to chemotherapy, specific inhibitors of EGFR tyrosine kinase activity (EGFR-TKi), such as erlotinib and osimertinib, have been developed and are currently in clinical use. However, these targeted therapies are systematically confronted with the development of resistance mechanisms that lead to cancer recurrence (1).

The small GTPase RHOB plays a key role in primary resistance to EGFR-TKi, as evidenced by the maintenance of the AKT kinase-dependent signaling pathway in response to treatment (2). To identify the mechanisms by which RHOB maintains cell survival, we performed a detailed analysis of the RHOB interactome in EGFR downstream signaling pathways by combining genetic screens using the split-GFP tripartite technology (3-5) and proteomic analysis of associated complexes. Our results revealed a set of protein partners of the MAPK and PI3K signaling pathways for which the functional and molecular links with the RHOB GTPase remain unknown. The main objective of the internship is to validate the identified candidates by biochemical (co-immunoprecipitation) and cellular (confocal imaging) analyses. The most valuable RHOB-partner complexes in terms of stability and impact on cell signaling will be further characterized by biophysical approaches.

Techniques : Co-immunoprecipitation, cell culture, transfection, RNA/CrispR interference, western blot, high-content imaging (Operetta CLS™), protein production and purification, SPR, SEC-MALS.

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