ROLE OF AKT PATHWAY IN THE PREDICTIVE RESPONSE TO ALLITINIB, A NEW GENERATION EGFR-INHIBITOR, IN A PANEL OF HNSCC CELL LINES.

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Introduction and Objectives: Epidermal growth factor receptor (EGFR) is overexpressed in 90% of head and neck squamous cell cancer (HNSCC) patients, turning EGFR inhibitors promising therapeutic drugs for HNSCC. Cetuximab was the first monoclonal antibody against EGFR approved for HNSCC. Once EGFR therapies have limited success, the development of irreversible tyrosine-kinase-inhibitors with pan-HER activity have been a highlighted strategy. In this work, we investigated the efficacy of Allitinib, an irreversible EGFR inhibitor, in a panel of HNSCC cell lines and analyzed its effect over intracellular pathways. Material and Methods: Combination therapy between anti-EGFR (Cetuximab or Allitinib) and anti-AKT(MK2206) was evaluated in 8 HNSCC cell lines through MTS. Growth inhibition score (GI) was ranked in highly-sensitive (HS), moderate-sensitive (MS) and resistant (R). Mutational status of EGFR, KRAS, NRAS, PIK3CA and PTEN genes was determined by sequencing. EGFR amplification was evaluated by FISH. Effect of AKT1 on drug response was analyzed by gene silencing. Viability, cytotoxicity and apoptosis were evaluated through ApoTox-Glo and migration rate was measured by wound healing assay. Results: Mutational analysis revealed EGFR mutation(p.H773Y) associated with gene amplification (EGFR:CEN7 >4) for HN13 cells and KRAS mutation (p.G12S) for JHU-28 cells. According to Cetuximab GI score, SCC25 cells were HS; SCC4 and Fadu were MS; JHU-12, JHU-28, HN13 and HCB289 were R. For Allitinib, SCC25, SCC4 and JHU13 were HS; Fadu and HCB289 were MS; HN13, JHU12 and JHU28 cells were R. Both therapies did not inhibit Akt phosphorylation in HN13 resistant cell line, but MK2206 combination restored Allitinib sensitivity. Additionally, AKT1 silencing showed decreased viability, increased cytotoxicity, activated caspases 3/7 and migration reduction rate upon Allitinib treatment. Conclusion: Allitinib presented greater cytotoxic profile when compared with Cetuximab. Importantly, the combination with AKT inhibitor restored Allitinib response in resistant cell lines, constituting an attractive therapeutic option for HNSCC patients.