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P2Y2 receptor plays a critical role in nucleotide mediated EGFR phosphorylation

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Purpose

Our goals were to investigate the differential phosphorylation of EGFR in response to nucleotide stimulation and determine the role of purinergic receptors on EGFR cross activation. Phosphorylation features, such as site occupancy and dynamics of modification at specific residues of proteins are known to affect protein stability and function.

Method:

The effect of individual nucleotides on cell migration after injury was monitored using a live cell migration assay every 20 min over 16 h. Real-time PCR was employed to investigate the expression of P2Y receptor mRNAs. To investigate the phosphoproteome profile of Human Corneal Limbal Epithelial (HCLE) cells following injury/nucleotide and growth factor stimulation, we performed mass spectrometry-based quantitative phosphorylation analysis. Stable isotope labeling of amino acids in cell culture (SILAC) combined with transfection of siRNA directed to P2Y receptors was used to test their role in nucleotide-induced phosphorylation of the observed sites.

Results:

UTP and UDP stimulation resulted in 100% wound closure, while ADP and ATP stimulation achieved 30% and 70% wound closure, respectively. Only P2Y2 mRNA exhibited a change over time following injury. Using mass spectrometry-based analysis of the phosphorylation sites, we have mapped and quantified a total of 20, 141 and 163 phosphotyrosine sites in control, ATP, and EGF treatment respectively. Quantitative analysis of the same data set using Progenesis LC-MS showed a differential phosphorylation profile in EGFR tyrosine residues. SILAC coupled with siRNA mediated knock down of specific receptors revealed a decrease in the extent of phosphorylation of EGFR-Y974, EGFR-Y1086, and EGFR-Y1148 in the P2Y2 knockdown cells. Our parallel Immunoblots blots using site-specific antibodies on cultures transfected with siRNA to P2Y2 and P2Y4 receptors showed a decrease in phosphorylation of EGFR-Y845, EGFR-Y1068, and EGFR-Y1086 residues.

Conclusion:

Our live cell migration results show that UTP stimulation did induce wound closure comparable to physiologic levels of EGF (0.5 nM). The response to UTP implicates the role of the P2Y2 and P2Y4 receptors that are supported by an increase in the expression of P2Y2 mRNA following injury. The lack of migration in response to ADP is supported by the minimal constitutive expression of the P2Y1 receptor. A decrease in expression of P2Y2 mRNA directly correlated with a decrease in phosphorylation of EGFR tyrosine residues following UTP stimulation. Together these suggest that activation of the P2Y2 receptor results in a specific phosphorylation pattern of EGFR that mediate the injury.