

論文名：

Investigation of signaling pathways regulating both cell motility and proliferation of cultured oral keratinocytes for therapeutic use (Abstract)

新潟大学大学院医歯学総合研究科

氏名 小林 亮太

---

Since quality control of cells for therapeutic use is necessary for consistent, high-quality, large-scale biomanufacturing, special attentions have been paid to an image-based technology enabling noninvasive and quantitative evaluation of cultured cells. We developed a unique image-based analysis using optical flow algorithm and found that the cell/colony motion correlated with the proliferative capacity of oral keratinocytes. However, how the cell motility and proliferative capacity of oral keratinocytes are regulated by signaling molecules is not elucidated. In this study, we found that the cell motility and proliferative capacity of oral keratinocytes was attributed to epidermal growth factor (EGF) in our culture system because EGF increased the motion speed of cells and decreased the population doubling time significantly. RNAi-mediated EGF receptor (EGFR) knockdown remarkably slowed down the cell motility of oral keratinocytes. Additionally, we revealed that the specific inhibitor of EGFR considerably suppressed the cell motility and proliferative capacity with phosphorylated-EGFR downregulation. Furthermore, Src inhibitor noticeably dropped the cell motility, but not proliferative capacity, with diminishing p-Src and p-EGFR. In contrast, although STAT3 phosphorylation and Erk1/2 inhibitors restrained phosphorylation of the substrates, neither reduced motility and proliferation. Finally, we showed that blocking of the p-Src and -EGFR promoted E-cadherin expression, consistent with cell growth pattern, suggested the activated EGFR and Src negatively regulated the cell-cell attachment. These data collectively demonstrate that EGF/EGFR axis and Src/PI3K/Akt/mTOR pathway play a major role in the cell motility and proliferative capacity of oral keratinocytes, indicating that this downstream cascade might serve as a potential target of pharmacological manipulation for quality control.