Multiplexed immunofluorescence imaging and single-cell analysis of MAPK and mTOR signaling in a large cohort of colorectal cancer subjects

Christopher Sevinsky, Yunxia Su, Alberto Santamaria-Pang, Vidya Kamath, Michael Gerdes, Qing Li, Fiona Ginty

General Electric Global Research Center. Molecular Imaging and Diagnostics Advanced Technologies Program, Niskayuna, NY 12399

High content in vitro cellular imaging and comprehensive single cell level analysis of model systems and clinical specimens are yielding significant gains in cancer research, but suffer from inherent limitations. To exploit the best properties of these approaches, we developed a novel immunofluorescence application that enables high order multiplexed analysis of FFPE tissue samples with low cost and resources. We performed a comprehensive functional analysis of mTOR (phospho-RPS6 and phospho-4E-BP1) and MAPK (phospho-ERK1/2) in colorectal cancer cells. Since the cancer sustaining activation of mTOR complex one (mTORC1) and MAPK mediated regulation of protein synthesis, we expected to find a high degree of correlation between RPS6 (Thr/Ser235/236) and 4EBP1 (Thr/Tyr37/46) phosphorylation at the cellular and subject levels. Surprisingly, we found that tumors exhibiting RPS6 phosphorylation were distinct from the vast majority of cells with mTORC1 mediated 4E-BP1 phosphorylation. Further analysis revealed that phosphorylation of these canonical mTORC1 downstream targets is frequently mutually exclusive in entire tumor stained sections. These results illustrate divergent growth signaling through mTORC1 and MAPK in human CRC tissues that may aid in the development and evaluation of therapeutics targeting these pathways.

Experimental Design

Subjects: 747 colorectal cancer patients – 43/60 targets in each patient

Multiplexed immunofluorescence: Directly conjugated Cy3, Cy5 and Cy7 labeled primary antibodies to biomarkers of colorectal cancer and tissue environment;

Sequential fluorescence microscopy: 38 rounds of imaging; 60 protein targets; 6 rounds of baseline autofluorescence

Automated image analysis: Registration; single cell and subcellular nuclear, cytoplasmic and membrane segmentation; target quantification

High content data analysis: 6 modules: cluster analysis of single cells using median phospho-S6, phospho-4E-BP1 and phospho-ERK1/2.

Abstract

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