

Quantitative co-imaging of RNA, protein, and nuclear architecture in single cancer cells

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Both intrinsic and extrinsic micro-environmental factors influence pathway function in individual cancer cells, which results in establishing tumor heterogeneity. We need tools to explore the heterogeneity in histological contexts; therefore, we have been developing imaging methods to allow simultaneous quantification of RNAs and proteins at the single cell level. Simultaneous visualization of the activation of these pathways and subsequent gene expression in single cells is needed to understand sensitivities and responses to drug treatment in cancer cells with cell-to-cell variability.

To quantify individual transcripts in breast cancer single cells, we first established single molecule fluorescent in situ hybridization (smFISH) technology using multiple short probes (20-nucleotide length) labeled with single fluorophores. Her2 mRNA particle counts are closely related to DNA copy number data in a variety of breast cancer cells. Unexpectedly, Her2 mRNA particles are enriched in the nuclei of both Her2+ and non-Her2+ cells, representing around 30 % of their total particles, and suggesting their potential subcellular RNA localization and consequent translational regulation. The nuclear Her2 mRNA aggregates present in diffraction-limited resolution imaging are resolved using super-resolution structural illumination microscopy (SR-SIM). Next, we established “immune-smFISH,” combining immunocytochemistry and smFISH for the simultaneous co-imaging of protein and mRNA, and applied it to time-lapse analyses of Her2 mRNA expression and phosphoAkt protein levels in Her2-positive breast cancer single cells treated with the HER-family tyrosine kinase inhibitor Lapatinib. Akt signaling activities down-regulated by Lapatinib treatments are recovered after 48 hrs, as previously reported by Western blot analysis. However, phosphoAks are not recovered in SKBR3 cells grown on glass coverglasses, as opposed to plastic, possibly due to extrinsic micro-environmental factors. Nuclear morphometries are also analyzed by measuring the size, intensity, aspect ratio, perimeter, roundness, and circularity of DAPI-stained nuclei, whose differences might cause expression level changes.

Our imaging methods provide information about the association among nuclear morphology, transcription level, protein expression, and subcellular localization in individual cells, and would be applied to pinpoint target cancer cells of aberrant signaling and subsequent end-point gene expression in human tumor biopsy samples and xenograft tissues.