Real-time monitoring of single-cell heterogeneity in HIF dependent gene transcription

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High-altitude exposure and anemia induce the transactivation of the erythropoietin (Epo) gene by hypoxia-inducible factor (HIF)-2. Sufficient oxygen activates prolyl-4-hydroxylase domain (PHD)-mediated hydroxylation of HIF-2α, causing its proteasomal degradation. Our group previously showed in mice that hypoxia leads to transcriptional bursts in renal Epo-producing (REP) cells, which occurs only in a subset of all potential Epo-producing cells. To better understand the transcriptional heterogeneity observed in this mouse model, we used multiplex mRNA in situ hybridization of Epo and other targets of the HIF pathway on the single-cell (sc) level in Kelly neuroblastoma cells. While the known high sc-heterogeneity of tumors is usually attributed to genomic alterations and/or microenvironmental variability, preliminary results demonstrated that there might also be a strong intrinsic cell-to-cell variability in hypoxic gene expression patterns, even in clonal cell lines. To study the longitudinal, real-time hypoxic induction of specific genes on the sc-level, two different visualization/reporter systems have been developed. On one hand, the CRISPR/Cas9-mediated integration of 24 PP7 stem-loop sequences (derived from *Pseudomonas aeruginosa*) into the gene of interest is used to visualize the binding of the fluorescently tagged PP7 coat protein (PP7-PCP system). On the other hand, *“cell access through RNA sensing by endogenous adenosine deaminase acting on RNA”* (CellREADR system) is based on a mRNA target-dependent translational switch between two reporter proteins by ADAR-mediated editing of stop codons. The CellREADR system worked well with an exogenously expressed mRNA template, where 53.5% of all transfected cells expressed the reporter protein. With endogenous β-actin mRNA as target, 85.4% of all transfected cells expressed the reporter protein. We are currently testing various mRNA targets such as Epo and PHD2/3 in cells exposed to HIF-stabilizing conditions. With these two methods we will be able to quantitate the sc-heterogeneity of hypoxia-inducible de novo transcription of HIF target genes, elucidating the unexpectedly high intrinsic heterogeneity of the hypoxic transcription pattern, even in clonal cells cultivated under identical culture conditions.