Underlying influences on the distribution pattern of renal EPO$^+$ cells

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Renal erythropoietin (EPO)-producing cells belong to the cell population of interstitial fibroblasts, which are characterized by a stellate shape and the expression of the marker PDGFR-β. EPO-producing cells are primarily located at the corticomedullary border. Under hypoxemic conditions, the number of EPO-producing cells increases from the corticomedullary border towards the cortex surface. However, genetic activation of the hypoxia signaling pathway by PDGFR-β cell-specific deletion of the von-Hippel-Lindau (Vhl) protein led to induction of EPO in most interstitial fibroblasts throughout all kidney zones. Interestingly, EPO induction independent of oxygen availability using pharmacological PHD-inhibitors (PHDi) mirrors the physiological expression pattern. Thus, we aimed to analyse possible reasons why EPO expression is mainly restricted to the renal cortex despite the presence of potential EPO-producing cells throughout the kidneys.

Firstly, mice with inducible PDGFR-β cell-specific PHD2, PHD3 and PHD2/PHD3 deletions were used to test the hypothesis that interstitial subpopulations may exist that differ in their PHD expression, especially PHD3 expression, and thus determine the physiological expression pattern. In addition, EPO/PDGFRβ-PHD coexpression was analysed under different conditions using multiplex RNAscope. On the other hand, we investigated whether there are zonal differences regarding the sensitivity of HIF-2 stabilization and the subsequent activation of other HIF-2α target genes such as regulator of G-protein signaling 4 (RGS4) and adrenomedullin. Thus, kidneys of mice under different conditions (PHD deletions, severe anemia, single/multiple administration of a PHDi) were analysed.

While PHD3 deletion alone had no effect on renal EPO levels, codeletion of PHD2/PHD3 led to EPO levels (6000 EPO cells/section) that were more than twice as high as those in the kidneys of PHD2 deficient mice (2600 EPO cells/section). In both mouse models, EPO$^+$ cells could be detected evenly across the cortex, the outer medulla and partly in the inner medulla. The density of EPO$^+$ cells per kidney zone, however, was higher after PHD2/PHD3 deletion compared with PHD2 deletion alone. In parallel, PHD3/PDGFR-β coexpression showed no obvious differences in regard to its zonal distribution. Consistent with the overall EPO expression pattern in kidneys of PHD2/PHD3 deficient mice, nuclear HIF-2α protein staining could be detected in interstitial cells of all kidney zones. In contrast, in kidneys of anemic (hematocrit < 25%) or PHDi (single dose) treated mice HIF-2α$^+$ stained fibroblasts could mainly be detected along the corticomedullary border and in the cortex. In parallel, induction of the HIF-2α target genes adrenomedullin and RGS4 was observed in EPO$^+$ cells but not in deeper regions of the outer medulla or inner medulla. Preliminary findings after multiple PHDi administration showed massive EPO induction (>3000 EPO cells/section) that was however also mostly restricted to the cortex and corticomedullary border.

Although our results show both PHD2$^+$ and PHD2/PHD3$^+$ fibroblast subpopulations, these are evenly distributed across all kidney zones. Therefore, these subpopulations cannot be solely responsible for the typical physiological EPO expression pattern. Furthermore, our results indicate that medullary fibroblasts, although expressing HIF-2α mRNA, seem to require even stronger or longer stimuli for HIF-2 stabilization than the here described physiological and pharmacological stimuli.