Hypoxia and iron imbalance impairs JMJD6-mediated histone demethylation of VHL in preeclampsia

Hypoxia and iron imbalance impairs JMJD6-mediated histone demethylation of *VHL* in preeclampsia

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Objectives - Chronic hypoxia is a defining feature of preeclampsia. Mounting evidence implicates the O_2 and Fe(II)-dependent Jumonji C domain histone demethylases (JmjDs) as epigenetic mediators of hypoxic gene expression. We recently identified the bifunctional oxygen sensor, JMJD6, as a regulator of VHL (von Hippel Lindau tumour suppressor) protein stability in the human placenta. JMJD6 plays a key role in the histone code by demethylating arginine residues on histones 3 (H3R2me2) and 4 (H4R3me2). Given that *VHL* is downregulated in preeclamptic placentae, we examined JMJD6 function as a histone demethylase in regulating *VHL* in preeclampsia.

Methods - Placentae were obtained from preeclamptic (PE; n=25), and normotensive age-matched control (AMC; n=21) pregnancies. For *in vitro* demethylation studies, histones isolated from primary cytotrophoblasts or PE and AMC placentae were incubated with recombinant JMJD6 enzyme at 3% or 8% O_2, followed by Western Blotting.

Results - Western blotting revealed significantly elevated H3R2me2 and H4R3me2 expression in PE placentae. JMJD6 enzyme demethylated both histone targets in AMC, but not PE placentae. Placental levels of the Fe(II) ferroxidase, ceruloplasmin were strikingly elevated in PE and, therefore, we hypothesized that reduced Fe(II) availability limits JMJD6 activity in PE. *In vitro* demethylation of primary cytotrophoblasts revealed decreased expression of both histone targets in 8%, but not in 3% O_2, indicating optimal demethylase activity in normoxia. Importantly, addition of excess Fe(II) partially rescued JMJD6-mediated demethylation in primary cells in hypoxia and histones from PE placentae, thereby highlighting the importance of iron in mediating JMJD6 function in preeclampsia.

Conclusion - This study provides novel evidence of interplay between iron availability and oxygen on JMJD6 demethylase function in the human placenta. The hypoxic environment in PE increases ceruloplasmin, impacting on Fe(II) availability. This, in turn, impairs JMJD6 demethylase activity, thereby contributing to the decreased *VHL* in PE.

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