Structural Characterization of Hypoxia Inducible Factor α - Prolyl Hydroxylase Domain 2 interaction through MD Simulations

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Introduction

Prolyl Hydroxylases (PHDs) are an enzymatic family regulating cell oxygen-sensing. Under physiological oxygen concentrations, PHDs hydroxylate the hypoxia-inducible factor α (HIFs-α) protein family allowing their recognition by the von Hippel-Lindau tumor suppressor protein (pVHL) and subsequent proteasomal degradation. Hypoxia inhibits PHDs activity, thereby stabilizing HIFs-α which initiate the transcription of genes involved in cell metabolism adaptation to hypoxia. As a main hallmark of cancer, hypoxia promotes abnormal angiogenesis, cell proliferation, and survival. The PHDs isoforms (PHD-1,2,3) are thought to have a variable and cell-dependent impact on tumor progression. All isoforms hydroxylate HIF-α (HIF-1,2,3α), however, with different affinities. What determines these differences and how they pair with tumor growth is poorly understood.

Methods

Here, molecular dynamics (MD) simulations and computational tools were used to identify and characterize the PHD2 substrate specificity. In particular, we investigated the binding property of PHD2 in complexes with HIF-1α and HIF-2α. We also characterized the role of the phospho-Thr405 (TPO) localizing on the PHD2 C-terminus in acting as a molecular switch. In parallel, conservation analysis and binding free energy calculations were performed for all complexes to better understand PHD2 substrate affinity.

Results

Our data suggest a direct association between the PHD2 C-terminus and HIF-2α that is not observed in the PHD2/HIF-1α complex. Further, our results indicate that phosphorylation of Thr405 (TPO) is causative of a variation in binding energy, albeit this PTM has only a limited structural impact on PHD2/HIFs-α complexes. In contrast, we observed a statistically significant difference in binding energy between complexes formed by PHD2/HIF-1α and PHD2/HIF-2α that pair with a number of inter-molecules interactions specific for each substrate. Collectively, our findings suggest a C-terminus role in modulating PHD2 substrate specificity.

Discussion

Our findings suggest that the PHD2 C-terminus may potentially act as a molecular regulator of PHDs activity. Further, the presence of a phororylation site in this region is suggestive of a

fine regulative switch. Our data show that specific conserved residues allow the enzimatic discrimination between different substrates, suggesting that mutations in these sites emerging in cancer may interfer with the binding of a single substrate, ideally conferring an adaptative advantage rather than compromising the entire PHD2 enzymatic activity.