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Functional implications of O-GlcNAcylation dependent phosphorylation at proximal site on keratin 18

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Keratins 8/18 (K8/18) are phospho-glycoproteins and form the major intermediate filament network of simple epithelia. Dynamic equilibrium of these phosphoglyco-proteins in the soluble and filament pool is an important determinant of their cellular functions, and is known to be regulated by site-specific phosphorylation. However, little is known about the role of dynamic O-GlcNAcylation on this keratin pair. We have demonstrated that O-GlcNAcylation of K8/18 exhibits a positive correlation with their solubility (Nonidet P-40: a mild detergent extractability). Heat stress, which increases K8/18 solubility, resulted in a simultaneous increase in O-GlcNAc on these proteins. Conversely, increasing O-GlcNAc levels were associated with a concurrent increase in their solubility. This was also associated with a notable decrease in total cellular levels of K8/18. Unaltered levels of transcripts and the reduced half-life of K8 and K18 indicated their decreased stability on increasing O-GlcNAcylation. On the contrary, the K18 glycosylation triple mutant (K18 S29A/S30A/S48A) was notably more stable than the wild type K18 hepatocytes. We further investigated the site-specific interplay between these two modifications in regulating the functional properties of K18, such as, solubility, stability and filament organization. The well characterised three O-GlcNAcylation (Ser29, Ser30, and Ser48) and two phosphorylation (Ser33 and Ser52) serine sites on K18 were further investigated. The hepatocyte line stably expressing site-specific single, double, and triple O-GlcNAc and phospho-mutants of K18 were used to identify the site(s) critical for regulating these functions. Keratin 18 mutants where O-GlcNAcylation at Ser30 was abolished (K18-S30A) exhibited reduced phosphorylation induced solubility, increased stability, defective filament architecture, and slower migration. Interestingly, K18-S30A mutants also showed loss of phosphorylation at Ser33, a modification known to regulate the solubility of K18. Further, the K18 phosphomutant (K18-S33A) mimicked K18-S30A in its stability, filament organization, and cell migration. These results indicate that O-GlcNAcylation at Ser30 promotes phosphorylation at Ser33 to regulate the functional properties of K18 and impact cellular processes like migration. Thus contrary to the present dogma that O-GlcNAcylation and phosphorylation on the same or adjacent sites on most proteins antagonize each other in regulating protein functions, our results demonstrated a positive interplay between these two modifications. In conclusion, our findings suggest that a novel, positive interplay between O-GlcNAcylation and phosphorylation at adjacent sites on K18 regulates its fundamental properties.

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