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PROTEOMIC ANALYSIS OF TARGETS OF SER65 PHOSPHORYLATED UBIQUITIN

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Recent studies have highlighted additional levels of complexity in the post-translational modification of proteins by ubiquitin (Ub). For example, Ub itself can be modulated by phosphorylation to act as a second messenger in PINK1-Parkin mediated mitophagy. However, the full physiological significance of Ub phosphorylation is unclear. Thus, the project sought to catalogue mammalian target proteins modified by covalent attachment of phosphoSer65-(poly)Ub.

Ub-WT and Ub-Ser65Asp (phosphomimetic mutant) sequences were engineered to allow overexpression of His/FLAG-tagged proteins in HEK293T cells. An additional Leu73Pro mutation was introduced to stabilise targets of Ub modification. Endogenous proteins modified by covalent attachment of transfected Ub sequences were purified by IMAC. Covalent modification was confirmed by western blotting and targets of modification were identified by LC-MS/MS.

Western blotting affirmed greater target protein modification by Ub-Ser65Asp and Leu73Pro mutants, the latter an indication of higher resistance to deubiquitination. Proteomic analysis suggested differential modification of various target proteins by Ub compared to Ub-Ser65Asp, including endogenous SUMO2. By transfecting GFP-SUMO2 and its C-terminal-GG deletion mutant, along with phosphomimetic Ub, we confirm that Ub-Ser65Asp modifies SUMO2, rather than vice versa. Finally, we confirm that transfected His/FLAG-SUMO2 is modified by endogenous phosphoSer65-Ub. SUMO2 represents a novel target of Ser65 phosphorylated ubiquitin.

The topics to be covered include targeted drug delivery, tumor therapies, and remote catheter navigation. It will be shown how iMRI enhances the safety and efficacy of these procedures.

BIOGRAPHY

Julius T Dongdem is currently a PhD candidate in the area of molecular cell biology and development at the University of Nottingham, UK. He has completed an MPhil degree in Biochemistry and Molecular Pharmacology at the University of Nottingham, UK. Julius had earlier obtained a BSc degree in Biochemistry and Chemistry and MPhil in Biochemistry and Molecular Medicine from the University of Ghana Legon, Ghana. He is a lecturer at the University for Development Studies, Ghana and has more than 10 articles to his credit in reputable Journals. He has also served in editorial board of more than seven Journals.

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