

# ADDENDUM

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In the paper by Yang *et al.* entitled “Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability” (*Nature Cell Biol.*, **8**, 1074–1083; 2006), we demonstrated that Ser 149 of p53 is modified with O-GlcNAc. The spectrum of the peptide we identified as being O-GlcNAcylated (Fig. 4c) has a unique MS-MS pattern, which has no conventional GlcNAc oxonium ions and shows a small difference in molecular weight at the modification site. For these reasons, we cannot draw a definite conclusion that the peptide was modified with intact O-GlcNAc based on the result presented in Fig. 4c alone. However, we can conclude that p53 was modified on Ser 149, because  $\gamma$  series ions from

positions 8–12 of peptide 140–156, which contained Ser 149, were detected as DTT-attached forms after BEMAD treatment (Fig. 4d). Furthermore, biochemical functions were changed when Ser 149 was mutated to Ala. Phosphorylation on Thr 155 and ubiquitination of His-p53 S149A were not reduced by treatment with streptozotocin, an O-GlcNAcase inhibitor, although both phosphorylation and ubiquitination of His-p53 WT was dramatically reduced (Figs 5, 6). Recently, we found additional O-GlcNAc modification sites on p53 using deletion and point mutation experiments. This finding can explain why the O-GlcNAc western blot band of His-p53 S149A did not completely disappear in Fig. 5b.