

## Ubiquitination is indispensable for the insulin-sensitising activity of the adaptor protein APPL1

KKY Cheng<sup>1</sup>, KSL Lam<sup>1</sup>, Y Wang<sup>1</sup>, WD Zhu<sup>1</sup>, A Xu<sup>1,2</sup>

<sup>1</sup>Department of Medicine, The University of Hong Kong, Hong Kong

<sup>2</sup>Department of Pharmacology, The University of Hong Kong, Hong Kong

**Background:** Insulin inhibits hepatic glucose production through activation of the protein kinase Akt. Our previous work has identified the multi-domain adaptor protein APPL1 as a positive modulator of insulin-evoked Akt activation in hepatocytes. However, the detailed mechanisms remain elusive. This study aimed to address the role of post-translational modification in APPL1-mediated potentiating effects on hepatic actions of insulin.

**Methods:** Rat hepatocytes were infected with adenovirus encoding luciferase control or FLAG-tagged APPL1 together with HA-tagged ubiquitin, followed by serum starvation and stimulation with insulin (10 nM). Total cell lysate was subjected to immunoprecipitation, Western blot analysis, and real-time quantitative PCR analysis. Ubiquitination of APPL1 was detected by Western blot analysis. Ultracentrifugation was employed to separate the hepatocytes into cytosolic and plasma membrane fractions.

**Results and Conclusion:** In rat hepatocytes, APPL1 undergoes ubiquitination upon insulin stimulation in a time-dependent manner. APPL1 ubiquitination is lysine 63-linked but not lysine 48-linked, indicating that this post-translational modification may regulate its cellular localisation and functions, but is not responsible for proteasomal degradation. Our mutagenesis experiment identified that APPL1 residue lysine 160 is the site for its ubiquitination. Mutation of lysine 160 to arginine abolishes the potentiating effects of APPL1 on insulin sensitivity. Further analysis reveals that an E3 ubiquitin ligase, tumour necrosis factor receptor associated factor (TRAF6), is responsible for ubiquitination of APPL1. This E3 ligase is associated with APPL1 upon insulin treatment. Over-expression of TRAF6 further enhances insulin-stimulated APPL1 ubiquitination. Moreover, knockdown of TRAF6 expression attenuates insulin-mediated translocation of APPL1 from cytosol to cellular membrane, which in turn inhibits the potentiating actions of APPL1 on insulin signalling. Taken together, these results support the notion that APPL1 ubiquitination is a vital step for hepatic functions of insulin through modulating the intracellular trafficking of Akt.

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## Increasing prevalence of hypertension in Hong Kong Cardiovascular Risk Factor Prevalence Study: role of general and central obesity

BMY Cheung, KL Ong, AWK Tso, TH Lam, KSL Lam

Department of Medicine, The University of Hong Kong, Hong Kong

**Introduction:** General obesity and central obesity are well-known risk factors of hypertension. We investigated the change in the prevalence of hypertension in the population-based prospective Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) and the relationship of change in blood pressure with change in body mass index (BMI) and waist circumference over a follow-up period of 11.9 years.

**Methods:** A total of 2888, 1942 and 1798 subjects in CRISPS-1 (1995-1996), CRISPS-2 (2000-2004) and CRISPS-3 (2005-2008) were included in this analysis respectively. Hypertension was defined as blood pressure  $\geq 140/90$  mm Hg or taking anti-hypertensive medication. General obesity was defined as BMI  $\geq 27.5$  kg/m<sup>2</sup> and central obesity was defined as waist circumference  $\geq 90$  cm in men or  $\geq 80$  cm in women.

**Results:** The prevalence of hypertension increased from 18.1% to 39.4% ( $P < 0.001$  after adjusting for age and sex). The prevalence of central obesity increased from 25.4% to 41.4%, but that of general obesity decreased from 16.8% to 14.8% (both  $P < 0.001$  after adjusting for age and sex). Among 1347 subjects who did not take any anti-hypertensive medication at both CRISPS-1 and CRISPS-3, the change in waist circumference, but not that in BMI, was associated with the changes in both systolic and diastolic blood pressures (beta=0.087,  $P=0.015$  and beta=0.122,  $P < 0.001$  respectively).

**Conclusions:** The increase in prevalence of hypertension might be explained by the increase in central obesity. Our findings further confirm the importance of waist circumference in this population; calculating the BMI alone may give a false sense of security.

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