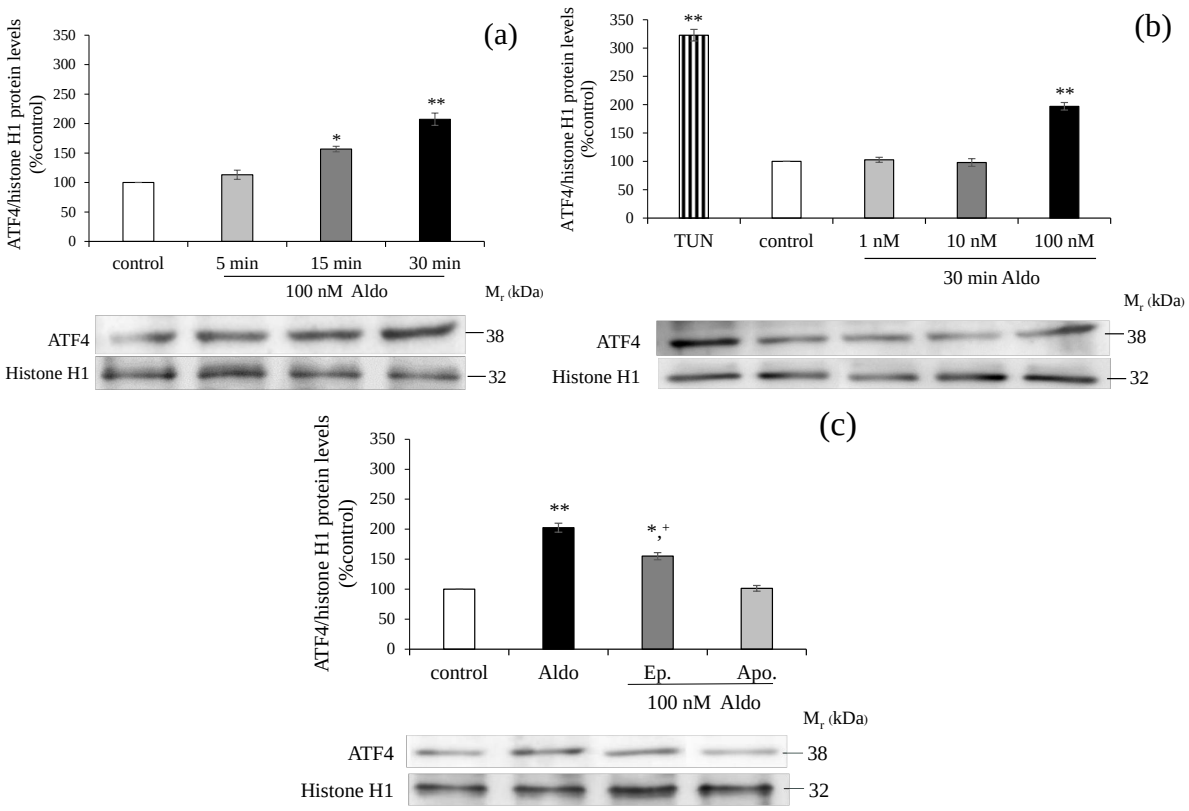

Fig. 3 (a) Aldosterone elevated nuclear protein content of ATF4 in a time-dependent manner. (b) After 30-min incubation, only the dose of 100 nM aldosterone significantly enhanced nuclear ATF4 protein level. (c) Aldosterone induced ATF4 protein accumulation through MR dependence and NADPH oxidase activation. Histogram bars show densitometric analysis ratios of ATF4 to histone H1 intensity, and the representative immunoblot photographs are presented. Data are means ± SD of 4 independent experiments. * $p < 0.01$ compared with the control group, ** $p < 0.001$ compared with the control group, + $p < 0.01$ compared with the Aldo group. Tunicamycin (TUN, 2 μg/ml) was used for a positive control.

indicate that, in the MR pathway, aldosterone has the potential stimulation on p47phox which is an adaptor protein to regulate the assembly of the NADPH oxidase complex. Regarding the mechanism of aldosterone in this milieu, aldosterone in the MR pathway exerts p47phox-induced NADPH oxidase activation and stimulates AT_1R dimerization in human renal PTEC cells.

The present study provides the first in vitro data which showed aldosterone increases nuclear ATF4 protein abundance in a time-dependent manner at the dose of 100 nM aldosterone (Fig. 3a). ATF4, a transcription factor responded to ER stress induced by unfolded protein, plays a role in the regulation of downstream genes and proteins involved in the maintenance of protein homeostasis in the ER [16, 31]. In a previous study, it has been reported that homocysteate, an oxidative stress inducer, increased nuclear ATF4 protein accumulation in cultured mouse cerebral cortex [17]. Aldosterone has also been reported to nongenomically elevate oxidative stress in porcine proximal tubular cells and in Madin Darby canine kidney cells (representing cells of distal tubule) [32]. Furthermore, aldosterone has been shown to rapidly enhance oxidative stress through NADPH oxidase [14]. These data suggested that aldosterone might contribute to the accumulation of nuclear ATF4 protein through activation of the NADPH oxidase pathway. Nevertheless, the effect of nuclear ATF4 protein accumulation by aldosterone has not been examined. In the present study, we provide evidence that aldosterone-induced nuclear ATF4 protein accumulation is mediated through NADPH oxidase activation since the accumulation was abolished by apocynin, an inhibitor of NADPH oxidase, (Fig. 3c). Collectively, aldosterone nonge-

nomically activated NADPH oxidase through an MR-dependent pathway that may induce ER stress and consequently stimulated nuclear ATF4 protein accumulation in HK-2 cells.

In conclusion, the present in vitro study in human renal PTECs demonstrates that aldosterone nongenomically increases AT_1R dimerization and nuclear ATF4 protein accumulation through MR and NADPH oxidase. Our findings s uggest t hat, in proximal tubular cells, aldosterone induces AT_1R dimerization and activates endoplasmic reticulum stress.

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