CONTRIBUTION OF P62 TO THE PHENOTYPE TRANSITION OF CORONARY ARTERIAL MYOCYTES FROM MICE LACKING CD38 GENE

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BACKGROUND

- CD38 plays a critical role in autophagosome trafficking and fusion with lysosomes, thus controlling autophagic flux in coronary arterial myocytes (CAMs) under atherogenic stimulation (*Cardiovasc Res* 2014; 102: 68-78).
- CAMs from CD38$^{-/-}$ mice fed with the Western diet exhibited phenotypic changes towards a more dedifferentiated state and abnormal extra cellular matrix metabolism. However, the mechanism by which this CD38 gene deficiency produces phenotype transition is largely unknown.
- The signaling adaptor p62 is critical in the activation of transcription factor NF-κB, which could be accumulated by autophagy defect and thereby promotes smooth muscle cell proliferation and tumorigenesis (*Cell*. 2009; 137: 1062-1075).

The present study was designed to examine whether P62 contributes to the proliferation and phenotype transition of CAMs when CD38 gene was lacking or lysosomal function was blocked.

METHODS

- Animal models for atherogenic changes in coronary arterial wall or CAMs were produced, and primary cell culture of CAMs, Western blot analysis, and confocal microscopy of autophagic and dedifferentiation markers were conducted as we described previously (*J Cell Mol Med. 2014; 18: 2165-2175*).
- Cell cycle analysis and proliferation rate determination were performed as described in previous studies (*Plos One 2015; 10: D119506*)

RESULTS

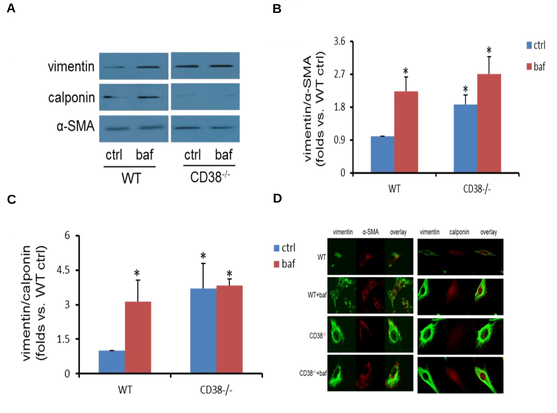

Fig. 1. Phenotype change induced by CD38 gene deficiency or lysosomal inhibition. It was shown that CD38 gene deficiency or lysosomal inhibition with bafolimycin (Baf) induced significant increase in the ratio of vimentin to calponin or α-SMA in CAMs (Panel A-C). Confocal microscopy also confirmed increased vimentin, but decreased calponin and α-SMA (Panel D). These results indicate the marked transition from contractile to synthetic phenotype of CAMs with CD38 gene deletion or lysosome function inhibition.

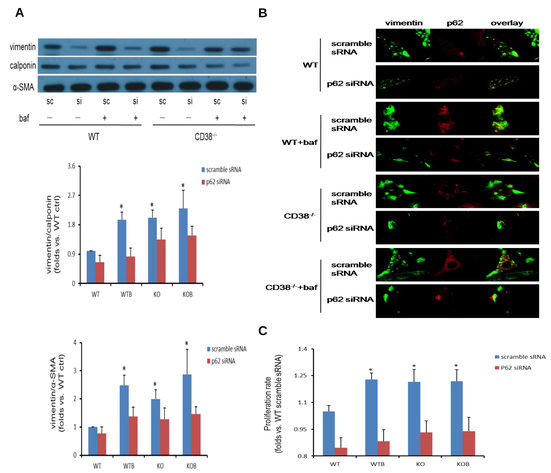

Fig. 2 P62 mediates proliferation and phenotype transition of CAMs due to CD38 gene deficiency or lysosomal inhibition. We transfected CAMs with p62 siRNA and measured proliferation rate and protein content of vementin. It was shown that p62 gene knockdown reversed the phenotype change and proliferation of CAMs induced by CD38 gene deficiency or lysosomal inhibition.

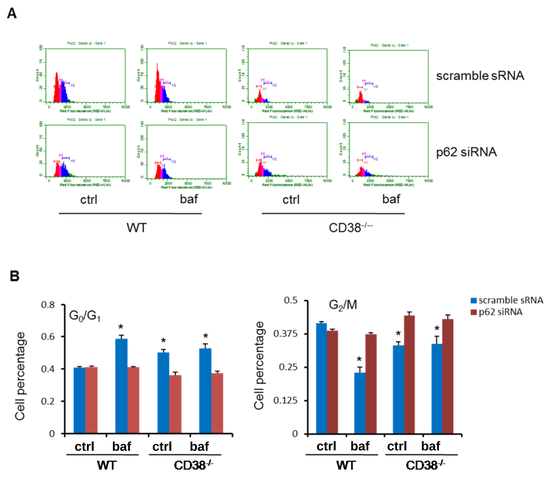

Fig. 3. CD38 gene deficiency or lysosomal inhibition caused cell cycle change associated with increased p62. In CAMs with CD38$^{-/-}$ or baf treatment, percentage of cell in G_2/M phase decreased, while CAMs in G_0/G_1 phase increased. These changes in cell cycle could be recovered by p62 siRNA, indicating that p62 accumulation prevents G_2/M arrest and promotes the cell to enter into mitosis.

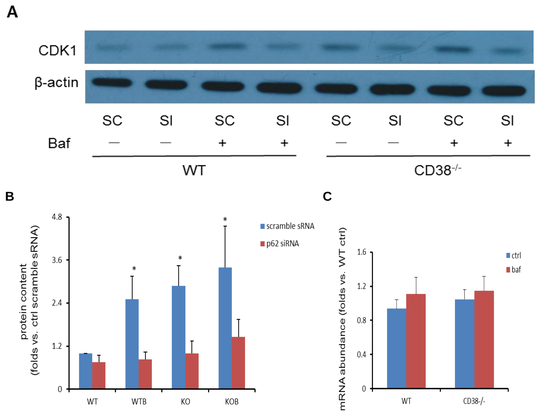

Fig. 4. Protein and mRNA levels of CDK1 in WT or CD38$^{-/-}$ CAMs with or without p62 gene knockdown. We measured mRNA or protein levels of the cyclin-dependent kinase 1 (CDK1), a critical cell cycle regulator. We found CD38$^{-/-}$ or baf treatment could increase protein, but not mRNA level of CDK1. The p62 siRNA transfection blocked this changes in CDK1 levels in CAMs.

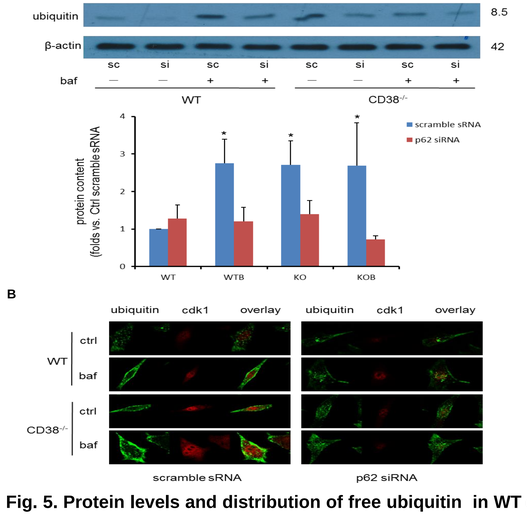

Fig. 5. Protein levels and distribution of free ubiquitin in WT or CD38$^{-/-}$ CAMs with or without lysosomal inhibition. Since CDK1 is mainly degraded through ubiquitin pathway, we analyzed the ubiuitin level and its binding activity. It was shown that free ubiquitin (not bound to other proteins) was increased and moved to cell membrane by CD38 gene deletion or baf treatment. It seems that a low degradation rate of CDK1 due to its compromised binding capacity to ubiquitin leads to increases in the level of CDK1 protein, but mRNA.

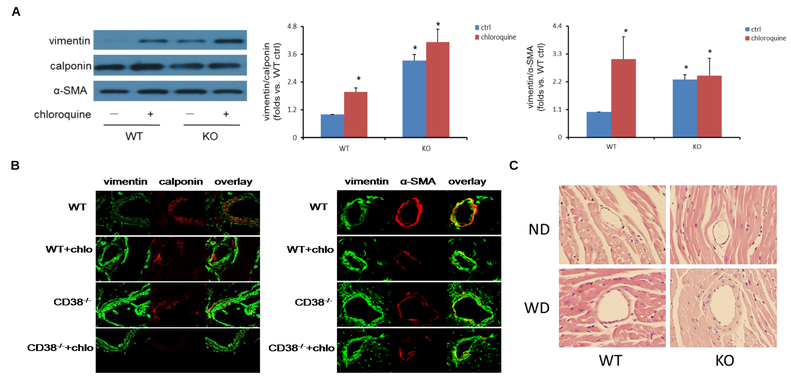

Fig. 6. Phenotype change of CAMs and Thickening of coronary artery wall in CD38$^{-/-}$ mice. We also conducted animal experiments to test the role of CD38 gene and lysosome function in the regulation of CAMs phenotypes. A significant phenotype transition was found in CAMs of CD38$^{-/-}$ mice, as shown by increased vimentin protein, but decreased calponin or α-SMA using isolated cells (A) or fluorescent staining of the arterial wall (B). This phenotypic transitional change was also observed in mice treated by chloroquine (chlo), a lysosomal function inhibitor. We also found significant thickening of the coronary artery wall in mice with CD38 gene deletion or treatment of chlo.

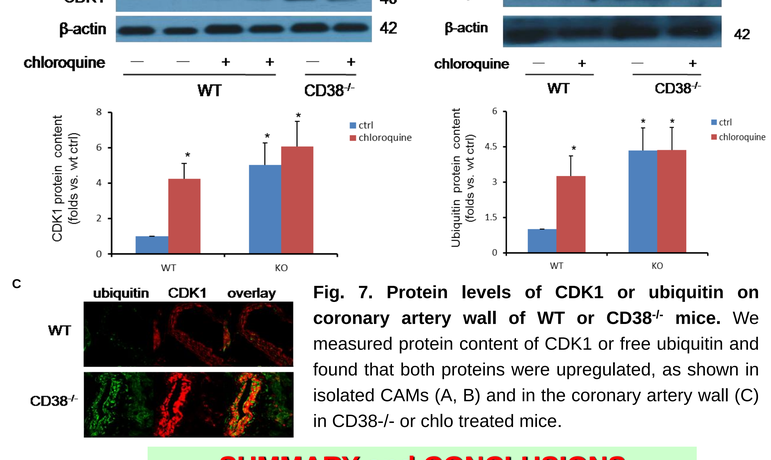

Fig. 7. Protein levels of CDK1 or ubiquitin on coronary artery wall of WT or CD38$^{-/-}$ mice. We measured protein content of CDK1 or free ubiquitin and found that both proteins were upregulated, as shown in isolated CAMs (A, B) and in the coronary artery wall (C) in CD38-/- or chlo treated mice.

SUMMARY and CONCLUSIONS

- CD38 gene deletion-associated lysosome dysfunction induced the accumulation of p62, leading to dedifferentiation and proliferation of CAMs.
- Inhibition of p62 accumulation attenuated dedifferentiation and proliferation due to lysosome dysfunction in CAMs.
- Compromised ubiquitination and degradation of CDK1 occurred due to lysosome dysfunction-induced dedifferentiation and proliferation of coronary arterial myocytes.

It is concluded that CD38 gene and its product importantly determine the phenotype of CAMs by its critical role in the regulation of autophagy, p62 level and CDK activity.