Low expression of autophagy-related protein 5 (ATG5) leads to suppression of autophagy in patients with Diabetic nephropathy & retinopathy

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Background and aims

Autophagy is a catabolic mechanism that involves lysosomal-dependent degradation of unnecessary or dysfunctional intracellular components. Autophagy plays role in many biological processes, including diabetic nephropathy (DN) and diabetic retinopathy (DR). Autophagy-related gene 5 (ATG5) is one of the most important participants in the autophagy mechanism. Deficiencies in ATG5 protein levels are associated with several diseases by influencing the level of autophagy pathway. Our study's aim was to investigate if aberrant expression of ATG5 protein or Atg5 gene is associated with DN or DR

Research Hypothesis

We hypothesize that alteration in the ATG5 protein levels or Atg5 gene expression may associate with diabetic complication; diabetic nephropathy (DN) or diabetic retinopathy (DR).

Materials &Methods

The study included 120 human participants in 4 groups – Healthy, diabetic (DM), DN and DR; and 10 mice in 2 groups – healthy and DN. Human peripheral blood mononuclear cells (PBMCs) lysates and murine renal lysates were subjected to Western blot analyses of ATG5 and LC3-II (a specific marker for autophagy). Immunohistochemical analysis was performed on mice renal tissues.

Results

1. Alteration in ATG5 protein levels and tissue expression

1.1 Differences of ATG5 protein levels in diabetes with and without complication compared with controls

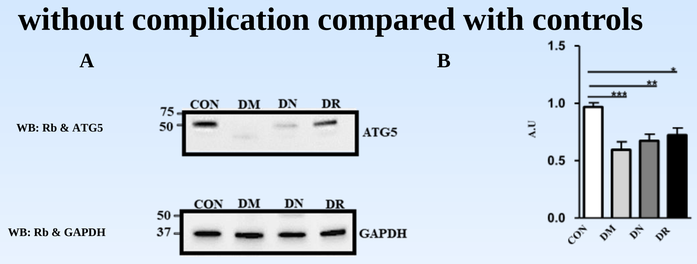

Figure 1.1 Alterations in ATG5 protein levels isolated from PBMCs of healthy controls and DM, DN, DR patients. A. Representative western blots of ATG5 protein level. PBMCs Protein samples were subjected to western blot analysis. **B.** Quantification of total western blot analysis. ATG5 protein levels were significantly higher in healthy control individuals compared with DM patients with and without complications. *p<0.05 **p < 0.01, ***p < 0.001.

1.2 Decreased ATG5 protein levels in mice kidney lysate

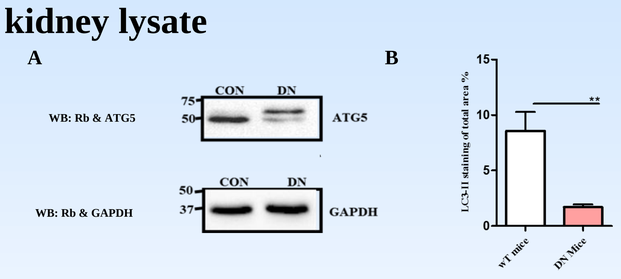

Figure 1.2 Renal ATG5 levels in WT and DN mice. A. Representative western blots of ATG5 protein levels in randomly selected mice renal lysates. **B.** Quantification of total Western blot. Renal ATG5 level was significantly reduced in DN renal lysate compared with WT. EM. Unpaired student's t-tests were performed to obtain the p values indicated on the graph **p < 0.01.

1.3 Decreased ATG5 protein expression in renal tissues of WT and DN mice

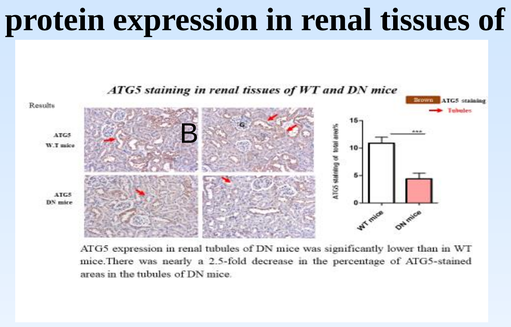

Figure 1.3 ATG5 expression in renal sections of DN and WT mice. A. Images show decrease of ATG5 expression (brown) in the tubules (red arrowheads) of DN mice compared with WT. **B.** Quantification of total ATG5 staining. The percentage of the stained area of ATG5 significantly decreased in at the renal tubules of DN mice compared with WT mice.

2. Reduce LC3-II levels is associated with lower level of autophagy in diabetes

2.1 Low levels of LC3-II in PBMCs of diabetic patients with and without complication

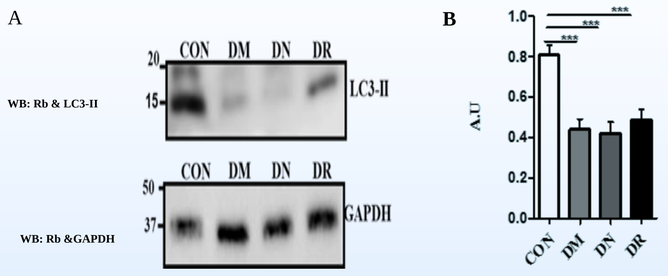

Figure 2.1 Reduced LC3-II protein levels in PBMCs of DM, DN, and DR. A. Representative western blots of LC3-II protein were randomly selected and represent one subject from the different groups. protein levels were significantly reduced in DM patients with and without complication compared to healthy control..

2.2 Low expression of LC3-II in DN mice kidneys

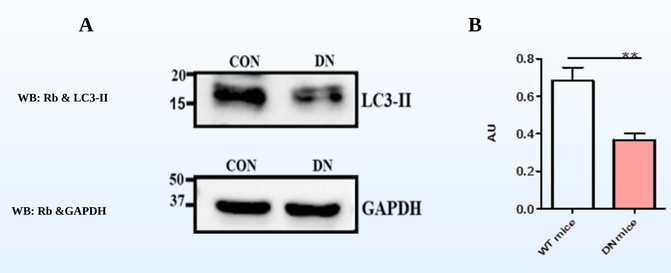

Figure 2.2 LC3-II renal protein levels in WT and DN mice renal lysates. A. Representative randomly selected western blots of LC3-II protein**. B.** Quantification of total Western blot. LC3-II renal level was significantly reduced in DN renal lysate compared with WT renal lysate.

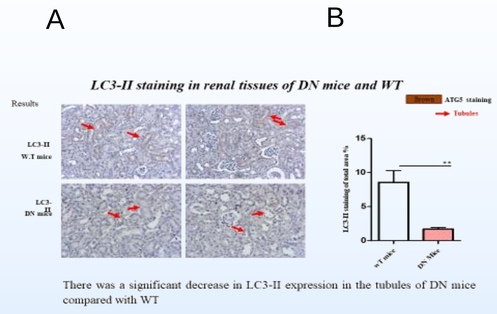

Figure 2.3 LC3-II staining in renal tissues sections of DN and WT mice. A. LC3-II immunostaining images of WT mice and DN. A significant decrease in LC3-II expression (brown) in the tubules (indicated by red arrowheads) of DN mice compared with WT. **B.** Quantification of LC3-II stained area percentage is markedly decreased in the renal tubules of DN mice compared with WT mice..

Conclusions

1. **Our findings indicate that ATG5, as well as its downstream collaborator LC3-II, are often down-regulated in diabetic patients, which contributes to deficiencies in autophagy protective process. Impairment of this process can lead to accumulation of abnormal proteins and damage molecules that can lead to the development and progression of DN &DR.**
2. **Therapeutic potential of ATG5 modulations as a novel treatment strategy for DN or DR patients through the autophagy Pathway.**
3. **ATG5 may serve as a goal in the development of drugs for diabetic and its complications.**