Letter to the Editor

Response letter: "Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b"

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In this issue of *Translational Cancer Research*, He *et al.* have intensively discussed our recent diabetes publication titled "*Novel lncRNA Erbb4-IR promotes diabetic kidney injury in db/db mice by targeting miR-29b*". We appreciate the insightful comments from the experts and would like to share our opinions here.

Increasing evidences showed that noncoding RNAs (ncRNAs) play a critical role in a variety of diseases (1,2), where long noncoding RNAs (lncRNAs) are defined as a class of longer than 200 nucleotides without protein-coding capacity. Recently, a number of studies have demonstrated the importance of lncRNAs on the regulation of gene expression and protein activity through diverse mechanisms (3). In contrast to microRNAs, lncRNAs are highly tissue- and cell-type specific (4), they have emerged as a new era of gene therapy. In our previously studies, we identified a number of novel lncRNAs associated with kidney diseases by targeting Smad3/TGF-β1 signaling (4-6), which is considered as one of the most important pathogenic pathways in renal fibrosis (7,8). In this work, we revealed one of the novel Smad3-dependent lncRNAs, Erbb4-IR, was largely increased in db/db mice associated with the progression of type-2 diabetic nephropathy (T2DN) and specifically triggered by advanced glycation end products (AGEs) in the cultured mouse tubular epithelial cells (mTECs) and mesangial cells (MMCs). More importantly, inhibition of renal Erbb4-IR protected db/db mice against T2DN development. Mechanistic study further identified this novel profibrotic lncRNA directly inhibit the expression of renoprotective miR-29b at transcriptional level via physical binding. Thus, we identified that Erbb4-IR has a pathogenic role in renal fibrosis under diabetic conditions and may represent as a therapeutic target for diabetic kidney disease.

Regarding the concerns from He *et al.*, ultrasound-microbubble-mediated gene transfer system is a well-established platform for studying biological functions of ncRNAs in our laboratory (5,9,10). According to our results, the system can kidney-specifically modulate gene expression peaked at day 7 after one application and declined since day 14. Three Erbb4-IR silencing treatments were conducted on db/db mice every 18 days since 12-week-old that lasted 8 weeks in total, resulted in a significant suppression of renal Erbb4-IR level in the treatment group compared to the empty vector control showing by ISH and real-time PCR analysis. Although the CRISPR-Cas9 genome-edited system offers several advantages over conventional methods (11), it would not only knockout the lncRNA, but also affect integrity of the host gene sequence i.e., Erbb4 of Erbb4-IR. As a recent study indicated Erbb4 deletion accelerates renal fibrosis (12), we prefer to inhibit Erbb4-IR specifically at RNA level.

In this study, we found that diabetic condition dramatically triggered Erbb4-IR induction in the nucleus of mesangial and epithelial cells with some cytosolic expression, whereas trace amount of Erbb4-IR is detected in the renal epithelial cells of db/m mice. Thus, binding of

Erbb4-IR on 3' UTR of miR-29b genomic sequence may affect the transcription efficiency of the immature miR-29b. Experiment was conducted to show that Erbb4-IR binding decreases miR-29b, this suppressive effect was cancelled after removal of Erbb4-IR binding site on miR-29b genomic sequence showing by reporter assay. Indeed, increasing evidences suggested the regulatory role of 3' UTR on gene transcription, which was also demonstrated as an inhibitory mechanism of TGF-β1/Smad3 signaling on natural killer cell development, where activated Smad3 physically bound on the 3' UTR of a master transcription factor E4BP4 to inhibit its mRNA production therefore largely suppressing NK cell development in the cancer microenvironment (13).

Nevertheless, Zhou's group also concerned about the renoprotective effects of miR-29b in diabetic kidney injury. Indeed, miR-29b is one of the well-documented anti-fibrotic ncRNAs in a number of diseases, loss of renal miR-29b was associated with progressive diabetic kidney injury, including microalbuminuria, renal fibrosis, and inflammation. In our previous study, we found that miR-29b was largely downregulated by AGEs associated with up-regulation of collagen matrix in mesangial cells via a TGF-β1/Smad3-dependent mechanism. More importantly, we further demonstrated the therapeutic effect of miR-29b on T2DN, which is capable of inhibiting progressive renal inflammation and fibrosis in db/db mice via restoring renal miR-29b level by the ultrasound-microbubble-based gene therapy (10).

In conclusion, we identified the pathogenic role of Erbb4-IR in T2DN, which may represent as a novel therapeutic target for diabetic kidney disease. However, understanding the molecular function and working mechanism of lncRNAs is still challenging due to its diversity and complexity (14). At present, lncRNAs are suggested as a competing endogenous RNA (ceRNA) or a molecular sponge for modulating the expression and biological functions of miRNAs (15), which is further supported by our study. In addition, the potential of lncRNAs to be used as biomarkers as well as therapeutics in clinical practice is still under debated. Thus, investigations should be continued to provide rationales for further development of lncRNA-based therapy into clinical settings.

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Footnote

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