

Post-Transcriptional Regulation of *edn1* mRNA in Kidney Collecting Duct Cells

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Regulation of endothelin-1 (ET-1) is thought to occur primarily at the level of transcription in a wide variety of tissues (Stow, 2011). However, the rate of mRNA turnover may also be subject to regulation, and the *edn1* mRNA seems to be particularly labile in kidney tissue samples. Several mechanisms affecting mRNA stability are known, such as the presence of AU-rich elements (AREs) and microRNA (miRNAs) regulation. Previous work in other labs has demonstrated the *edn1* mRNA levels can be affected by either type of regulation. However, these studies did not address these modes of regulation in the kidney, where the level of ET-1 expression is thought to be highest. We hypothesize that post-transcriptional mechanisms may account for the lability of *edn1* mRNA in the kidney. Endogenously produced miRNAs regulate gene expression either by inducing mRNA degradation or by repression of protein translation. In order to look for potential miRNA binding sites, the 3' untranslated region (UTR) of the *edn1* mRNA was examined. Strong miRNA binding sites were predicted in the 3' UTR of the *edn1* mRNA by two separate algorithms (Targetscan.org, microRNA.org). These analyses identified a single site where both miR-98 and the miRNAs of the let-7 family are predicted to bind. This putative binding site in the *edn1* 3' UTR is highly conserved across a wide variety of vertebrate species. Additionally, the miRNA database (microRNA.org) provided in silico evidence that miR-98, let-7c, and let-7f expression occurs in murine renal collecting duct tissue. The presence of these miRNAs in a cell line derived from a murine inner medullary collecting duct (mIMCD-3) was confirmed using a TaqMan® MicroRNA Assay. The mature forms of both let-7c and let-7f were present in abundant levels, while miR-98 was also found in only moderate levels. A luciferase reporter assay system based on pmirGLO has been designed to study the effects of mRNA regulatory elements in the *edn1* mRNA 3' UTR in mIMCD-3 cells. Preliminary data shows that the incorporation of the *edn1* 3' UTR results in an approximate 80% reduction in luciferase activity. A series of mutations in the 3' UTR have been constructed to either delete sections of the 3' UTR or to specifically alter putative miRNA binding sites and AREs in order to determine the mode of *edn1* mRNA regulation in kidney cells.

Stow LR, Jacobs ME, Wingo CS, Cain BD (2011). Endothelin-1 gene regulation. FASEB J 25: 16-28.