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POLY(ADP-RIBOSE) POLYMERASE-14 INTERACTS WITH TRISTETRAPROLIN TO SELECTIVELY REGULATE TISSUE FACTOR MRNA STABILITY: A NOVEL ROLE FOR ADP-RIBOSYLATION IN REGULATING MRNA TURNOVER AND THROMBOSIS

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Background Targeting post-transcriptional pathways is now emerging as a promising therapeutic strategy in a wide spectrum of diseases. Monocyte-derived tissue factor (TF) plays critical roles in atherothrombosis, but little is known about its post-transcriptional regulation. Tristetraprolin (TTP) is the most widely studied mRNA-binding protein that binds to the 3'UTR of target mRNAs and promotes degradation. Its function is negatively regulated by p38 MAPK. Poly (ADP-ribose)-polymerase-14 (PARP-14) belongs to a family of ~17 proteins with a PARP domain that generates negatively charged poly (ADP-ribose) adducts on intracellular proteins a post-translational modification implicated in diverse cellular functions.

Objective We sought to determine the roles for TTP and PARP-14 in regulating TF expression, and the potential role of ADP ribosylation in regulating TF mRNA turnover.

Methods/Results Compared to WT macrophages TF mRNA and protein expression were increased in $TTP^{-/-}$ and $PARP\text{-}14^{-/-}$ macrophages (both $p<0.05$). This was paralleled by an increase in TF mRNA stability in $TTP^{-/-}$and $PARP\text{-}14^{-/-}$ macrophages (both $p<0.001$). TF mRNA, activity and protein were increased *in vivo* (heart, lung, kidney, aorta and circulating leukocytes) in $PARP\text{-}14^{-/-}$ vs WT mice ($p<0.05$). Intravital microscopy demonstrated a 66% reduction in median arteriolar occlusion time in LPS-stimulated $PARP\text{-}14^{-/-}$ vs WT mice following ferric-chloride injury ($p=0.008$). RNP immunoprecipitation and RNA biotin pulldown assays demonstrated an interdependency for PARP-14 and TTP to form a ternary complex with TF mRNA. p38 inhibition reduced TF mRNA stability in WT but not in $TTP^{-/-}$ or $PARP\text{-}14^{-/-}$ macrophages. Inhibition of PARP activity reduced TF mRNA stability in WT macrophages ($p=0.018$), but not in $PARP\text{-}14^{-/-}$ macrophages. Interestingly, PARP-14 conferred selectivity for TTP to degrade TF mRNA, as PARP-14 deficiency had no effect on TNFα, an established target of TTP, both *in vitro* and *in vivo*. Accordingly, PARP inhibition had no effect on TNFα mRNA decay.

Conclusions These data define novel roles for TTP and PARP-14 in the selective regulation of TF mRNA turnover. PARP-14 functions as an accessory RNA-binding protein which is required for TTP to bind and degrade TF mRNA. These data indicate that both p38-mediated phosphorylation and PARP-14-mediated ADP-ribosylation are required to inhibit TTP-mediated TF mRNA decay. These data also show for the first time how the actions of TTP may be selectively regulated. Thus selective destabilization of TF mRNA via pharmacological inhibition of PARP-14 may offer a novel therapeutic strategy in atherosclerosis and cardiovascular disease where thrombosis is linked to inflammation-induced TF expression.