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MicroRNA-467g inhibits new bone regeneration by targeting Ihh/Runx-2 signaling☆

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ABSTRACT

MicroRNAs are important post transcriptional regulators of gene expression and play critical role in osteoblast differentiation. In this study we report miR-467g, an uncharacterized novel miRNA, in regulation of osteoblast functions. Over-expression of miR-467g inhibited osteoblast differentiation. Target prediction analysis tools and experimental validation by luciferase 3′ UTR reporter assay identified Runx-2 as a direct target of miR-467g. Over expression of miR-467g in osteoblasts down regulated Runx-2 and Ihh signaling components. Furthermore, silencing of miR-467g was done to see its role in Ihh and Runx-2 mediated bone healing and regeneration in a drill hole injury model in BALB/c mice. Silencing of miR-467g led to significant increase in new bone regeneration and Ihh and Runx-2 localization at injury site in a day dependent manner. In conclusion, miR-467g negatively regulates osteogenesis by targeting Ihh/Runx-2 signaling. We, thus, propose that therapeutic approaches targeting miR-467g could be useful in enhancing the new bone formation.

1. Introduction

MicroRNAs (miRNAs) are small (~22 nucleotides), single-stranded non-coding RNAs found in diverse organisms which have emerged as important post transcriptional regulators of gene expression (Dong et al., 2013; Eskildsen et al., 2011; Hassan et al., 2012; Inose et al., 2009; Li et al., 2009). MiRNAs negatively regulate translation of specific mRNAs by base pairing with partially or fully complementary sequences in target mRNAs and play a key role in various biological processes (Dong et al., 2013; Eskildsen et al., 2011; Hassan et al., 2012; Inose et al., 2009; Li et al., 2009). The expression of miRNAs is altered in various diseases like cancer, hepatitis C infection, myocardial infarction, and metabolic disease. For instance, miR-17–192 is significantly over expressed in lung cancer while let-7 is a tumor suppressor miRNA and aberrant expression of *let-7* results in oncogenic loss of differentiation (Christopher et al., 2016). MiRs also regulate several properties of cardiac physiology. The examples include miR-29, miR-30 and miR-133 which are down regulated in atrial fibrillation. On the other hand, miR-328 and miR-499 are up regulated in atrial fibrillation (Santulli et al., 2014). MiR-21 is substantially increased in response to cardiac injury in experimental murine models. Inhibition of miR-21 exhibits antihypertrophic and antifibrotic effects, which leads to a significant functional improvement (Thum et al., 2008). Other examples include miR-130a which is up regulated in hepatitis C virus infection. Introduction of anti-miR-130a in hepatocytes increased IFITM1 expression with concomitantly reduction in HCV replication (Shrivastava et al., 2013).

Many miRNAs have been identified which either negatively regulate osteoblast differentiation or bone formation by targeting osteogenic factors or positively by targeting negative regulators of osteogenesis (Bae et al., 2012; Dong et al., 2013; Eskildsen et al., 2011; Gao et al., 2011; Hassan et al., 2012; Inose et al., 2009; Li et al., 2009). These include miRNAs such as 133 and 204/211 which attenuate osteoblast differentiation by directly targeting Runx2 in C2C12 mesenchymal progenitor cells and MSCs respectively (Huang et al., 2010; Li et al., 2008) or miR-141 and miR-200a which target Dlx5 to inhibit osteoblast differentiation (Itoh et al., 2009). On the contrary, miR-335-5p directly targets and down-regulates Wnt inhibitor DKK1, enhances Wnt signaling and promotes osteogenesis (Zhang et al., 2011). Besides, several microRNAs including the *miR-34* family have been implicated in osteosarcoma tumorigenesis via their effects on notch signaling components (Nugent, 2015).

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