Chapter 7

Gene expression profiling reveals hsa-miR-128 affects metabolism
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7.1. Preface

In the previous chapter, we elucidated the molecular mechanism of hsa-miR-128 induced apoptosis with the help of transcriptomics. There are reports in literature which suggest one microRNA can target multiple functionally related genes and thus influence many physiological processes together. Hence, we further set out to explore our data obtained by gene expression profiling and looked for the other biological processes which get affected by hsa-miR-128.

7.2. Results

7.2.1. hsa-miR-128 Regulates Cholesterol and Fatty Acid Metabolism

Analysis of gene expression profiling data by ingenuity pathway analysis (IPA) further unpinned the hidden functions of hsa-miR-128. Mechanism of gene regulation by peroxisome proliferators via PPARα, hormone receptor regulated cholesterol metabolism, LXR (liver X receptor)/RXR (retinoid X receptor) activation were found to be enriched among the top toxicology functions (Fig. 7.1 A). Fatty acid metabolism, PPAR signalling and LXR (liver X receptor)/RXR (retinoid X receptor) activation were among the top five in canonical pathway list (Fig. 7.1 B).

7.2.2. Validation of Transcriptomics Data

Li et al. in 2007 showed that SIRT1 positively regulates LXR by deacetylation and alters fatty acid metabolism (Li et al., 2007). To check whether miR-128 had any effect on LXR and its targets (ATP-binding cassette ABC transporters ABCA1, ABCG1, ABCG5) which are known to promote the efflux of cholesterol from cells, we did real time PCR. We found miR-128 mediated reduction of SIRT1 decreased the mRNA levels of LXRβ, RXRα, ABCA1, ABCG1 and APOE and increased the mRNA levels of ABCG5 (promotes cholesterol excretion into bile) (Fig. 7.2 A).
Fig. 7.1: hsa-miR-128 regulates cholesterol and fatty acid metabolism. A. The top five toxicology functions assigned to the dataset by IPA software are represented here with their respective p-value. B. X-axis represents the top canonical pathways identified by IPA based on differentially expressed genes whereas y-axis (log value) represents the number of genes from the dataset that map to the pathway divided by the number of all genes ascribed to the pathway. The yellow line represents the threshold of p<0.05 as calculated by Fischer's test.
miRNA 128 regulates cholesterol and fatty acid metabolism. A-B. Real-time PCR analysis was performed as described in materials and methods (Chapter 2) for mRNAs of Liver X Receptor (LXR) pathway genes (A) and sterol regulatory element-binding protein (SREBP) pathway genes (B) in miR-128 overexpressing cells. 18s rRNA was used for the normalization. Graphs represent fold change in mRNA level. (n=3±SD; *p<0.05). C. Western blot analysis of SREBP1 and SREBP2 after 24 hours of transfection of 4 µg p(128) and negative control in HEK293T cells. β-actin bands are the loading control. Lower panel shows the graph which represents the fold change in the protein level as compared to the untransfected HEK293T cells. D. Real-time PCR analysis was performed for mRNAs of fatty acid synthesis genes in miR-128 overexpressing cells. 18s rRNA was used for the normalization. Graphs represent fold change in mRNA level. n=3±SD; *p<0.05 versus negative control. U=Untransfected, p(128)=HEK293T cells transfected with 4 µg p(128), N=negative control.
Several lines of evidences also suggested that SREBP2 (sterol regulatory element-binding protein) is a transcription factor that is generally found in balance with LXR and increases cholesterol uptake and synthesis by inducing the expression of genes like LDLR, HMGCS1 and HMGCR (Krycer and Brown, 2011), hence we next assessed the effect of hsa-miR-128 on mRNA levels of these genes. Transfection of HEK293T cells with hsa-miR-128 increased the mRNA levels of SREBP2, LDLR, HMGCS1, decreased the mRNA levels of SREBP1 but did not cause any change in the levels of HMGCR (Fig. 7.2 B). We further checked the expression of SREBP1 and SREBP2 at protein levels and found decrease in SREBP1 levels and increase in SREBP2 levels by western blotting (Fig. 7.2 C). Since SIRT1 has been found to regulate lipid homeostasis by positively regulating nuclear receptor PPARα and its co-activator PGC-1α (Purushotham et al., 2009), we checked their mRNA levels and observed significant reduction in PPARα and PGC-1α after hsa-miR-128 overexpression (Fig. 7.2 D). These results provided us some clues that apart from inducing apoptosis, hsa-miR-128 may be regulating cholesterol homeostasis and fatty acid metabolism.

7.2.3. Bioinformatic Analysis Revealed different Targets of hsa-miR-128 in Metabolism ABCA1 to be the Target of hsa-miR-128

We further set out to unpin the targets of hsa-miR-128 involved in fatty acid or cholesterol metabolism using 3 widely-used software, miRanda (John et al., 2004), RNAhybrid (Rehmsmeier et al., 2004) and Target Scan (Lewis et al., 2003), to avoid the overprediction. We found that the transcripts (ENST00000374736) of ABCA1, (ENST00000481739) RXRα, (ENST00000262735) PPARα and (ENST00000558518) LDLR harboured the hsa-miR-128 binding site in its 3’UTR. These are the key genes involved in cholesterol and fatty acid metabolism, hence role of hsa-miR-128 in regulating cholesterol and fatty acid metabolism needs further investigation.
7.3. Discussion

In addition to its deacetylase activity on transcription factors and other proteins, SIRT1 can also indirectly modulate critical players involved in lipid metabolism such as LXR (Li et al., 2007), PPAR-α (Purushotham et al., 2009) and co-activator PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α) (Rodgers et al., 2005). SIRT1 also deacetylates and inhibits sterol-regulated element-binding transcription factor (SREBP) (Ponugoti et al., 2010; Walker et al., 2010). Literature further suggests that SREBP2 can activate the transcription of HMG-CoA synthase (HMGCS1), HMG-CoA reductase (HMGCR), LDL receptor (LDLR) and LXR can activate ABCA1 and ABCG1 (Bengoechea-Alonso and Ericsson, 2007). Our current data showed that miR-128 reduces the transcript levels of LXRβ, RXRα, ABCA1 and ABCG1 but increases ABCG5 transcript levels. Decrease in RXRα and ABCA1 could be explained by the presence of binding sites of miR-128 in their 3’UTR as predicted by various target prediction algorithms. We also found that miR-128 increases the transcript levels of SREBP2, LDLR and HMGCS1 and reduces the transcript levels of SREBP1 but there was no change in the levels of HMGCR. We further showed that miR-128 through downregulation of SIRT1 reduces the transcript levels of PPARα and PGC-1α. Prediction of miR-128 binding sites in 3’UTR of PPARα by target prediction algorithms further adds a new layer of complexity to its regulation. A better understanding of how miR-128 regulates SREBP regulated pathways will probably be an important area of research in future. Considering the fact that SIRT1 alters genes of the cholesterol and fatty acid metabolism, our findings provide important evidence for the potential role of hsa-miR-128 in metabolism besides apoptosis. Therefore, hsa-miR-128 can be explored further as therapeutics for metabolic diseases besides cancer.