Localization of microRNA-375 in perinatal rat pancreas

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1 Introduction

MicroRNA-375 (miR-375, miR-375-3p) is by far the most highly expressed miRNA of pancreatic beta cells constituting up to 40% of beta cell miRNAs [1, 2]. Unsurprisingly, decreasing miR-375 levels have marked effects on beta cell phenotypes [3, 4], such as decreased glucose-stimulated insulin secretion and beta cell mass, while transgenic overexpression does not cause diabetes [5, 6]. Moreover, forced expression of miR-375 markedly impairs beta cell differentiation from embryonic stem cells [7].

The perinatal period is characterized by a number of metabolic changes and maturation of the endocrine system. In rats, the time of birth is characterized by a transient rise in beta cell replication as well as beta cell neogenesis followed by functional maturation of the endocrine pancreas. Consequently, a considerable increase in beta cell number occurs between embryonic day 20 (E20) and postnatal day 2 (P2) [8, 9]. As a major part of this beta cell expansion occurs by neogenesis from progenitors that may be located outside the endocrine compartment [10, 11], it is equally important to characterize the entire pancreas tissue in the perinatal period.

MiR-375 is highly enriched in endocrine cells [2], but is not completely restricted to islet cells: miR-375 is also expressed in the human hypothalamus, colon, small intestine and stomach (https://ccb-web.cs.uni-saarland.de/tissueatlas/patterns) [12]. The aim of the current study was to determine the relative localization of miR-375 in exocrine versus endocrine cells in rat perinatal pancreas and to investigate levels of miR-375 2 days before birth (E20), at the day of birth (P0) and 2 days after birth (P2).

2 Methods

2.1 Tissue-samples

Female Wistar rats, 10-11 weeks, were time-mated at Taconic, Denmark and transferred to local facilities one week prior to experiments. Animals had free access to...
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mg/ml yeast tRNA/1x Denhardt’s solution supplemented with 9.2mM citric acid if hybridization temperature was above 55°C). Complementary LNA probes (Exiqon) were DIG-labeled using DIG Oligonucleotide Tailing Kit 2nd Generation (Roche) according to manufacturer’s recommendations. 2.5pmol DIG-labeled probes were added to hybridization mix, heated to 90°C then iced, applied to each section and hybridized overnight at 22-25°C below probe Tm. Sections were washed at 12-15°C below probe Tm in decreasing SSC concentrations and incubated 15min at 37°C in 20μg/ml RNase A. For immunostaining sections were incubated 2hr in 1:100 anti-Digoxigenin-AP (Roche), Vulcan Fast Red was applied and nuclei were counterstained with Haematoxylin Carazzi. Sections were mounted from xylene and imaged on a Leica DM 4000 B using Leica Application Suite software. Negative controls included sections without probe and with scrambled

2.2 In situ hybridization (ISH)

Pancreata excised at E20, P0 or P2 were formalin-fixed and paraffin-embedded. Sections were denatured 10min at 42°C in 2.5mU/ml Proteinase K (Roche, Hvidovre, Denmark), incubated 5min in 4% paraformaldehyde, acetylated 10min in 0.1M triethanolamine pH 8.0/0.25% acetic anhydride and prehybridized for 1hr at 22-25°C below probe Tm in hybridization mix (50% formamide/5x SSC/0.5

Figure 1. In situ hybridization of perinatal rat pancreas for miR-375 (A-C) and a scrambled, negative control (D-F) in rat pancreas at day E20 (A, D), D0 (B, E) and D2 following birth (C, F). Vulcan Fast Red incubation results in bright red staining, and nuclei are counterstained with Haematoxylin (purple). Representative of 3-4 different pancreata per time point. Magnification: 400x.
control probe (Exiqon) that bear no homology to any known miRNA sequence.

3 Results

We measured, by in situ hybridization, the levels of miR-375 in rat pancreas at E20, P0 and P2 (Fig. 1, A, B and C). Negative controls included unsectioned sections (not shown) and sections hybridized with a scrambled LNA-spiked oligo (Fig. 1, D, E and F). MiR-375 was detected in islets on all three days, with minor change in intensity of the signal between these time points. Interestingly, the expression of miR-375 in exocrine tissue changed markedly over these 3 days (Fig. 1, A, B, C). MiR-375 levels at E20 were almost absent, while signal intensity at P0 was very high, and then decreased at P2. At all three time points, there was no discernible staining using a negative scrambled control oligo also with LNA substitutions. Moreover, no staining was observed in the absence of hybridization oligo (not shown).

4 Discussion

Our ISH data show that miR-375, in the rat perinatal pancreas, is also expressed in acinar cells. Although miR-375 has unaltered expression levels in islets during this period, the exocrine cells display a dynamic change in miR-375 levels, with almost undetectable miR-375 at E20, to prominent staining at D0 and also at D2 following birth. Thus, our data show that miR-375 is not restricted to islets cells in the pancreas, at least in rat perinatal pancreas. Moreover, the dynamic change of miR-375 localization in exocrine cells following birth suggest that miR-375 may regulate processes involved in the adaptation of the exocrine pancreas to digestion of milk.

Other factors have been shown to control the levels of miR-375, particularly in rodent islets; maternal gestational low-protein diet caused upregulation of miR-375 in islets of offspring at 3 weeks of age accompanied by decreased islet function and beta cell mass [13]. Since the phenotype of the global miR-375 knockout mouse also has decreased beta cells mass and beta cell numbers [4], it seems likely that the levels of miR-375 are precisely controlled physiologically.

MiRNAs have recently been shown to act as paracrine or close-to-endocrine signaling entities, where for example apoptotic beta cells via exosome can transfer active miRNAs [14] while insulin resistant tissues secrete microvesicles containing miRNAs, which can modulate beta cell function [15]. Thus, it is an attractive hypothesis that upregulated miR-375 in acinar cells following birth may modulate gene expression in acinar cells as well as in neighboring beta cells to mediate the marked beta cell proliferation in the period between birth and D2.

In conclusion, miR-375 is localized to both acinar and endocrine cells in rat pancreas following birth, whereas expression is more localized to islets at other time points.

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