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Published in:
Non-Coding RNAs in Endocrinology

DOI:
[10.1515/micrnado-2018-0001](https://doi.org/10.1515/micrnado-2018-0001)

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Larsen, L., Rosenstjerne, M. W., Nielsen, J. H., & Dalgaard, L. T. (2018). Localization of microRNA-375 in perinatal rat pancreas. *Non-Coding RNAs in Endocrinology*, 3(1). <https://doi.org/10.1515/micrnado-2018-0001>

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Research Article

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Localization of microRNA-375 in perinatal rat pancreas

<https://doi.org/10.1515/micrnado-2018-0001>

Received November 8, 2017; accepted January 23, 2018

Abstract: MicroRNA (miRNA)-375 is highly expressed in the pancreatic endocrine islets. Maintaining appropriate miR-375 levels is very important for beta cell development, function and proliferation. The aim of the current study was to investigate the regulation and localization of miR-375 in rat perinatal pancreas at embryonic day 20 (E20), postnatal day 0 (D0) and day 2 (D2). Expression levels of miR-375 were measured by in situ hybridization on fixed neonatal rat pancreas. Interestingly, while miR-375 was detectable at robust levels at all three time points, the major site of expression of miR-375 at D0 and D2 was in pancreatic exocrine cells. Our data show that miR-375 has a dynamic change of expression in pancreatic exocrine tissue during the perinatal period. Moreover, these findings indicate that pancreatic endocrine cells may not always be the major source of expression of miR-375 in pancreas. We suggest that the marked change of miR-375 levels in exocrine cells following birth could regulate processes involved in the adaptation of the exocrine pancreas to digestion of external nutrients derived from milk.

Keywords: Pancreas, perinatal, microRNA, miR-375, islets of Langerhans, beta cell, gene expression, exocrine pancreas, endocrine pancreas

Abbreviations: miRNA microRNA, ISH in situ hybridization, LNA locked nucleic acid, miRNA microRNA

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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

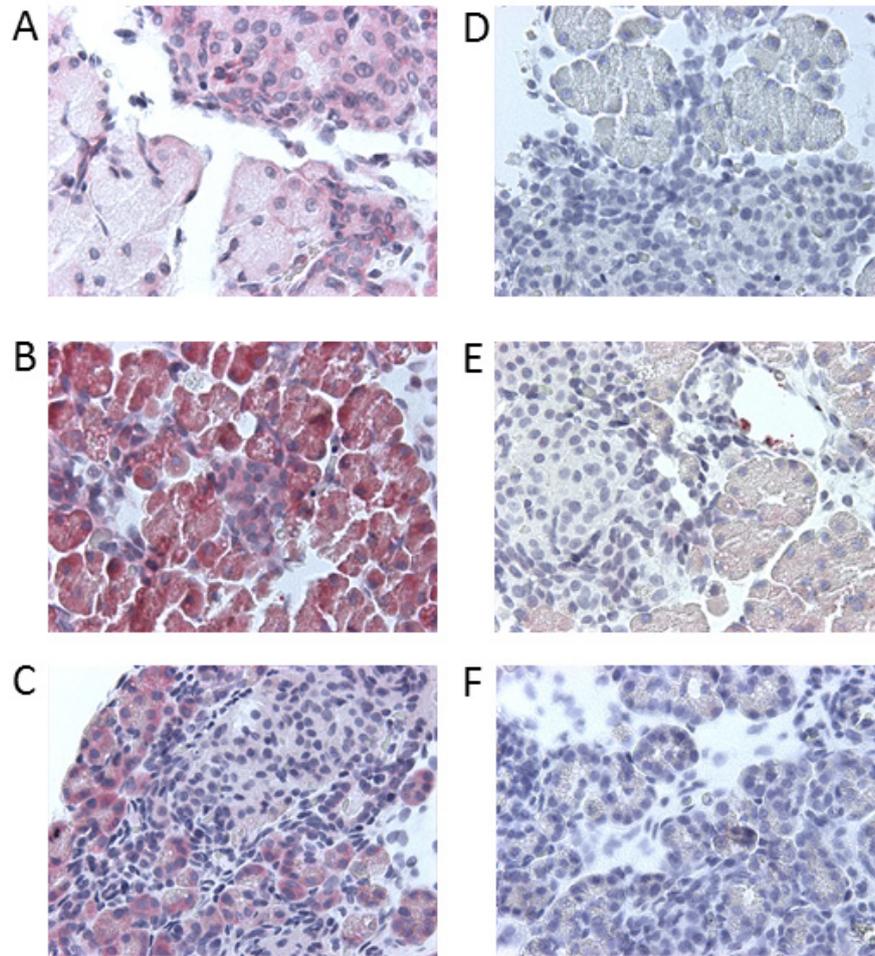


Figure 1. *In situ* hybridization of perinatal rat pancreas for miR-375 (A-C) and a scrambled, negative control (D-F) in rat pancreas at day E20 (A, D), D0 (B, E) and D2 following birth (C, F). Vulcan Fast Red incubation results in bright red staining, and nuclei are counterstained with Haematoxylin (purple). Representative of 3-4 different pancreata per time point. Magnification: 400x.

food and water and were kept on a 12hr light-12hr dark cycle. All studies were conducted in accordance with institutional guidelines and approved by the Danish Animal Experiments Inspectorate. The rats were killed at gestational day 20 (E20), immediately after birth (P0) or two days after birth (P2), and the offspring were decapitated.

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

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

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 unstained 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.

Acknowledgments: We are very grateful for the skilled technical assistance of Jacqueline Tybjerg and Susanne Sørensen. These studies were supported by the Danish Medical Research Council.

Conflict of interest statement: Authors state no conflict of interest

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