Identification of a LIN28B/mir-30a axis controlling thyroid cell invasion and proliferation

León Wert-Lamas[1], Garcilaso Riesco-Eizaguirre[1,2], Richard Gregory[3], Pilar Santisteban[1]

1. Endocrine Physiopathology Dept., "Alberto Sols" Biomedical Research Institute, Spain's High Science Research Council-Autonomous University Madrid (CSIC-UAM), Madrid, Spain. 2. Móstoles Universitary Hospital, Spain; 3. Stem Cell Program, Boston Children's Hospital, Dept of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY

Introduction

The oncofetal RNA-binding protein LIN28B plays an important role in differentiation and cancer progression. Lin28B induces epithelial-mesenchymal transition, and it is considered as a bad prognostic marker in several tumours. The aim of this work was to study the role of LIN28B on thyroid cancer progression.

In a first approximation a MiRNA targets computational predictions were performed with MiRanda algorithm and we found that, among others, both strands of mir-30a bind to the 3' UTR of LIN28B and several PI3K pathway effectors. As previous results, obtained by next-generation sequencing expression analysis performed in our laboratory, showed that mir-30a is one of the most significantly subexpressed mir in thyroid cancer, we decided to focus on its role over LIN28B and the PI3K pathway. We found that IGF-1 and RAS -PI3K pathway effectors- upregulated Lin28B expression. Overexpression of mir-30a resulted in LIN28B, HMGA2, H-RAS, PI3Kß, BCL-2 and CDK6 silencing, and in an increase in p27(Kip) protein levels in the nucleus. Inversely, LIN28B overexpressing cells showed a decrease in mir-30a levels and an increased expression of HMGA2 and H-RAS oncogenes. Furthermore, we observed that the LIN28B inhibition by mir-30a is specifically elicited through a direct binding to its 3'UTR. The general outcome was a significant decrease in invasion and proliferation in mir-30a overexpressing cells and, conversely, an increase in these parameters in LIN28B overexpressing cells. These data suggest the existence of a LIN28B/30a axis, with a double negative feedback regulation, whose tumoural shift, leading to overexpression of LIN28B and silencing of mir-30a, widely contributes to thyroid cancer progression.

Methods

PCCl3 and HRas-Doxiciclin inducible PCCl3 normal rat thyroid cell lines, and 8505c, Cal62 and NThy-ori human cell lines were used.

CMV-Lin28B and mir-30a precursor, and psiCheck2-Lin28B3'UTR were transfected with Lipofectamine 2000 for 48h before measurement. Invasion assays were performed with Corning® BioCoat™ Matrigel® Invasion Chambers.

IGF1 was added at 100 ng/ml

LIN28B protein expression is associated with BRAF and RAS mutations and Anaplastic Thyroid Carcinoma in human thyroid cancer cell lines

Fig. 1A)

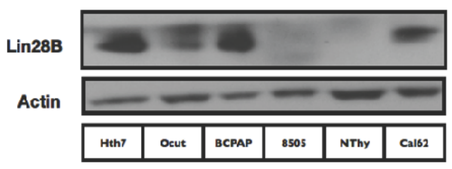


Fig. 1B)

	Cal62	Ocut2	Hth7	BCPAP	T238	Nthy-ori	H74	TPC	8505c
Lin28B	++	+	+++	++++					+
BRAF		MUT		MUT					MUT
RAS	MUT		MUT						
other	p53	PI3K	AKT	AKT	PI3K		p53	RET	

Fig. 1A) LIN28B protein Western Blot of a panel of human thyroid cancer cell lines total extracts. ß-Actin protein was used as a normalizer control

Fig. 1B) LIN28B mRNA levels and driver mutational status of a panel of human thyroid cancer cell lines. ß-Actin mRNA was used as a normalizer control

Mir-30a and LIN28B are reciprocally downregulated conforming a double negative feedback loop

Fig. 2A)

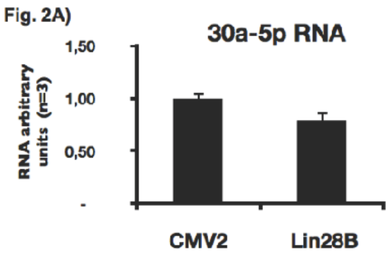


Fig. 2B)

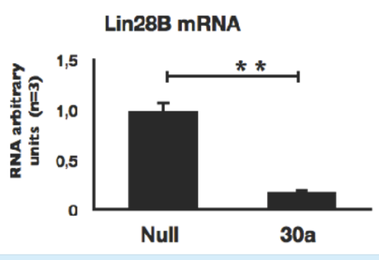


Fig. 2C)

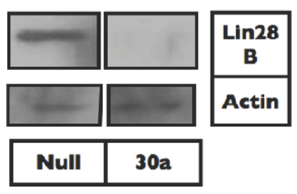


Fig. 2A) Mir-30a expression is repressed by LIN28B. RT-qPCR of RNA extracted of 8505c cells. Values represent a relative change in expression levels. **Fig. 2B)** LIN28B expression is repressed by mir-30a. RT-qPCR of RNA extracted of Cal62 cells. Values represent a relative change in expression levels. Each bar represents the average and SD of 2 independent experiments

Fig. 2C) LIN28B protein Western Blot of Cal62 cell line total extracts. ß-Actin protein was used as a normalizer control.

PI3K pathway effectors IGF-1 and RAS upregulate Lin28B expression

Fig. 3A)

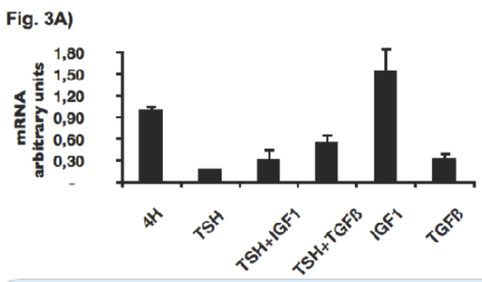


Fig. 3B)

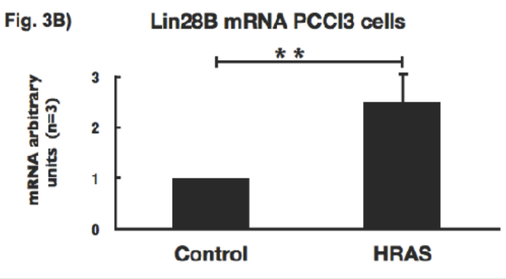


Fig. 3A) LIN28B expression is upregulated in presence of IGF1. RT-qPCR of RNA extracted of PCCl3 cells. Values represent a relative change in expression levels. **Fig. 3B)** LIN28B expression is induced by RAS activation. RT-qPCR of RNA extracted of PCCl3 cells. Values represent a relative change in expression levels.

Mir-30a represses HMGA2 and PI3K/AKT pathway effectors H-RAS, PI3Kß, BCL-2, CDK6 and upregulates p27; and LIN28B upregulates RAS and HMGA2

Fig. 4A)

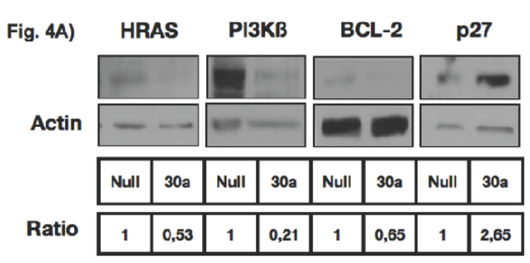


Fig. 4B)

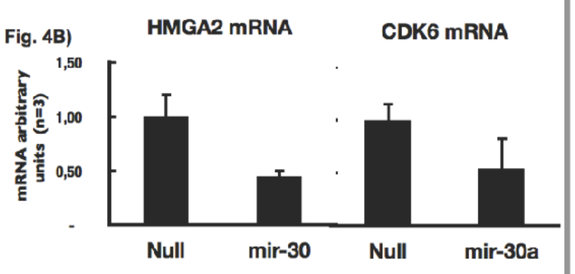


Fig. 4C)

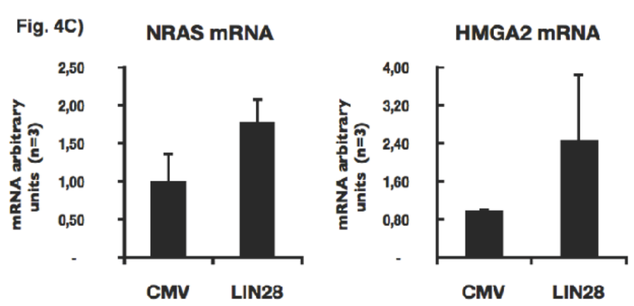


Fig. 4A) HRAS, PI3Kß, BCL-2 and p27 protein expression by Western Blot in mir-30a overexpressing Cal62 cell line total extracts. ß-Actin protein was used as a normalizer control. **Fig. 4B)** HMGA2 and CDK6 mRNA expression by RT-qPCR in mir-30-overexpressing Cal62 cell line. ß-Actin was used as a normalizer control. **Fig. 4C)** NRAS and HMGA2 mRNA expression by RT-qPCR in LIN28B overexpressing 8505c cell line

Mir-30a-driven LIN28B silencing is elicited by direct targeting with its 3'UTR

Fig. 5A)

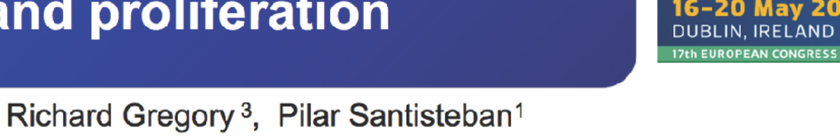


Fig. 5B)

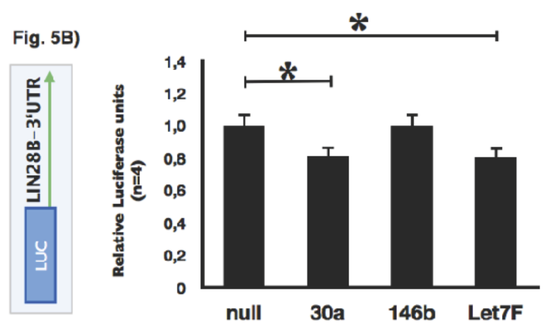


Fig. 5A) Mir-30a has 5 targets in LIN28B 3'UTR. Schematic representation of the multiple 30a binding sites in LIN28B 3'UTR.

Fig. 5B) Relative Luciferase activity in HeLa cells transiently co-transfected with psiCheck2-Lin28B-3'UTR 3'construct and pEGP-mir vectors Renilla was used as a transfection control normalizer. Each bar represents the average and SD of 3 independent experiments

LIN28B/30a axis controls invasion and proliferation in thyroid cell

Fig. 6A)

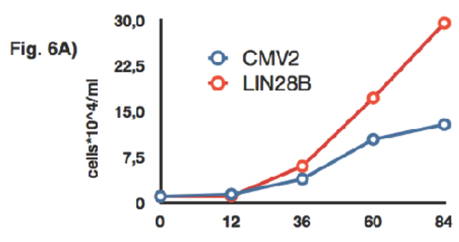


Fig. 6B)

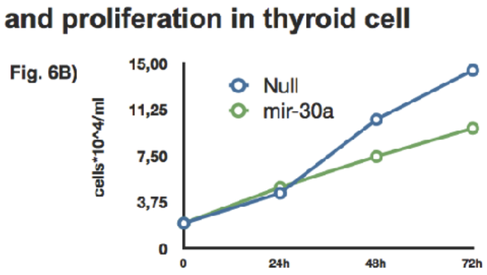


Fig. 6C)

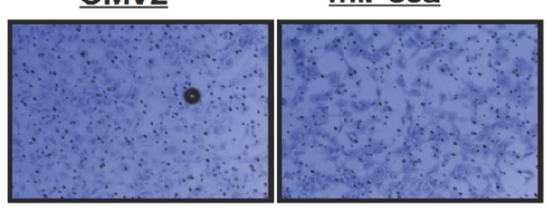


Fig. 6A) and 6B) Lin28B enhances proliferation and mir-30a slows it. Proliferation assay in 8505 and Cal62 respectively in cells transiently transfected with mir-30a and LIN28B.

Fig. 6C) Mir-30a inhibits invasion. Matrigel Invasion assays on Cal62 cells after 48h

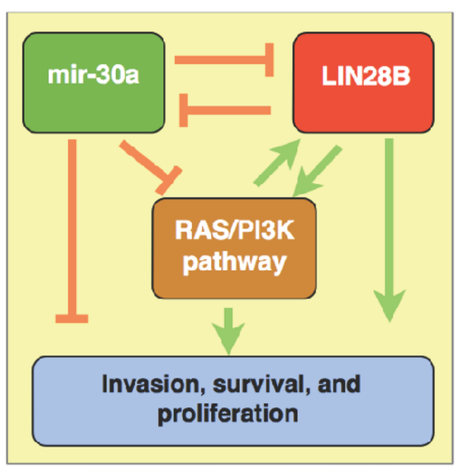
CONCLUSIONS

1. LIN28B protein expression is correlated with BRAF and RAS mutations and Anaplastic Thyroid Cancer, the most dedifferentiated and aggressive thyroid carcinoma.

2. Mir-30a represses LIN28B mRNA and protein levels and, inversely, LIN28B overexpression downregulated mir-30a levels, thus conforming a double negative feedback loop.

3. PI3K pathway effectors IGF-1 and RAS upregulate Lin28B expression

4. LIN28B overexpression increase expression of HMGA2 and H-RAS oncogenes. Conversely, mir-30a represses HMGA2 and PI3K/AKT pathway effectors involved in survival, motility and proliferation H-RAS, PI3Kß, BCL-2 and CDK6



5. Mir-30a-driven LIN28B silencing is elicited by direct targeting to its 3'UTR

6. LIN28B promoted -and mir-30a inhibited- proliferation and invasion in the thyroid follicular cell

7. LIN28B and mir-30a conform an axis, with a double negative feedback regulation, whose tumoural shift , leading to overexpression of LIN28B and silencing of mir-30a, widely contributes to thyroid cancer progression

References

1. Nam P et *al.*, Molecular basis for interaction of let-7 microRNAs with Lin28., Cell. 2011. 1080-1091
2. Zhong X et *al.*, Identification of microRNAs regulating reprogramming factor LIN28 in embryonic stem cells and cancer cells.J Biol Chem. 2010. 41961-71.
3. Johnson CD et *al.*, The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res. 2007. 7713-22.
4. Fuziwara CS et *al.*, MicroRNA Deregulation in Anaplastic Thyroid Cancer Biology. Int J Endocrinol. 2014.

DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY
PosterSessionOnline.com