DYSREGULATION OF MICRORNA BIOGENESIS IN DIABETIC SKIN

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BACKGROUND

- Clinical skin manifestations are common in diabetes, often being the first sign of the metabolic disorder (1).
- MicroRNAs (miRNAs), small non coding RNAs regulating gene expression at post-transcriptional level, have been found to play a role in skin homeostasis, including diabetic skin repair (2).
- We investigated whether miRNAs are differentially expressed in diabetic and non-diabetic skin.

Figure 1. General miRNA downregulation in the skin of diabetic mice

MiRNA profiling by microarray analysis (miRCURY LNATM) was performed on RNA extracted from the skin of streptozotocin-induced diabetic mice, a model of insulin-dependent type I diabetes.

- A) More than 400 different mouse miRNA species were identified; expression analysis revealed a general downmodulation in diabetic skin. In detail, among the 30 most significantly modulated miRNAs, 27 were downregulated and 3 were upregulated in diabetic mice.
- B-C) Validation by real-time qPCR of the differences observed in the expression of selected miRNA in the skin of a different group of streptozotocin-induced diabetic mice (STZ) or in the skin of db/db mice, a mouse model on insulin-independent type 2 diabetes (*p<0.05; **p<0.01).

Figure 2. Diabetes down-modulates miRNAs regulating pathways relevant for skin function

#	KEGG pathway	# genes	#miRNAs	p-value
1	LYSINE DEGRADATION	8	2	6.93 x 10 ⁻¹¹
2	AXON GUIDANCE	16	2	3.31 x 10 ⁻⁸
3	GAP JUNCTION	8	3	5.72x10 ⁻⁷
4	RETROGRADE ENDOCANNABINOID SIGNALING	12	2	1.30x10 ⁻⁶
5	TGF-BETA SIGNALING	9	4	3.49x10 ⁻⁶
6	LONG-TERM POTENTIATION	9	4	4.29x10 ⁻⁶
7	GASTRIC ACID SECRETION	9	4	2.17x10 ⁻⁵
8	GABAERGIC SYNAPSE	9	2	0.00017
9	GnRH SIGNALING PATHWAY	9	4	0.00018
10	WNT SIGNALING PATHWAY	14	5	0.00021

Pathway analysis using Tarbase showed an enrichment of signature-miRNA target genes also related to pathways playing an important role in the skin, such as TGF-β and Wnt signaling.

Figure 3. Reduced expression of genes involved in miRNA biogenesis in the skin of diabetic mice

From VN Kim et al, Nat Rev Mol Cell Biol, 2009

General reduction in miRNA expression has been correlated to reduced expression of genes involved in miRNA biogenesis (3). Therefore, we investigated whether miRNA alteration in diabetic skin associates with changes in the expression of Dicer1, DCGR8, Drosha, Exportin 5, and Ago2, involved at different levels in the miRNA biogenesis process (A).

B-C) All analyzed genes were expressed at significantly lower levels in the skin of streptozotocin-induced (STZ) and db/db mice, compared to non-diabetic controls.

D-E) Western blot confirmed reduced Dicer1 expression in the skin of diabetic mice (result from STZ mice is shown). (*p<0.05; **p<0.01)

Figure 4. Hypoxia down-modulates the expression of miRNA biogenesis genes

Besides being hyperglycemic, the diabetic skin environment is hypoxic due to microvascular impairment. Hypoxia has been found to down-regulate Dicer and Drosha expression in tumor cell types (4-5). Therefore, *in vitro* experiments with human dermal fibroblasts in hyperglycemic or hypoxic conditions were set up, and Dicer and Drosha expression analyzed.

- A-B) Hypoxia (250 μM CoCl₂, for 24 hours), but not hyperglycemia (35 mM D-glucose, for three weeks), induces Dicer and Drosha mRNA downregulation.
- C) Real time-PCR to check that the hypoxic culture conditions (250 μM CoCl₂ or 1% O₂, for 24 hours) are able to exert expected HIF1α mRNA decreased and VEGF-A mRNA increased expression.
- D) Scratch assay indicating that hyperglycemic fibroblasts manifest the expected impairment in migration.

CONCLUSIONS

These findings indicate a decreased expression of the majority of modulated miRNAs in diabetic skin associated to reduced levels of genes responsible for miRNA biogenesis, and suggest that miRNA dysregulation is linked to the hypoxic condition of diabetic skin.

References

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