MicroRNA Expression Signatures as potential Biomarkers in Cancer

Cara Martin, John O'Leary, Orla Sheils St. James' Hospital, Histopathology, Trinity College and Coombe Women's Hospital, Dublin, Ireland
Astrid Ferlinz, Simone Guenther Applied Biosystems, Applera Holding GmbH, Frankfurter Str. 129b, 64293 Darmstadt, Germany

ABSTRACT

In order to identify miRNA expression signatures associated with molecular triggers of oncogenesis, the expression profile of more than 150 human miRNAs in thyroid and cervical cancer cell lines was studied using Applied Biosystems TaqMan® MicroRNA Assays. We identified unique miRNA patterns associated with different pathological pathways. miRNA targets were predicted with web-based search algorithms. Multiple potential mRNA targets corroborated recent expression microarray data and the identified differentially expressed genes in the respective tumour samples.

INTRODUCTION

Cells have developed several safeguards to ensure a correct and coordinated cell division, differentiation and death. A defect in the regulatory factors, e.g. tumour-suppressor genes and oncogenes, will increase the probability of tumour development. miRNAs are a novel class of small non-protein-coding RNAs that function as negative gene regulators. They frequently control several target mRNAs resulting in diverse and complex biological processes. miRNAs regulate their mRNA target either by gene-silencing or by post-transcriptional repression of translation. Studies have suggested their involvement in processes such as cell proliferation, differentiation and apoptosis [1]. Since differential expression of miRNAs has been observed between malignant and normal tissues, they might serve as biomarkers for tumour diagnosis, the characterization of molecular pathways and the classification of disease subtypes.
Repressing the expression of important cancer-related genes, miRNAs might also turn out a valuable tool in the treatment of cancer [2].
a) Papillary Thyroid Carcinoma (PTC) is the most common thyroid malignancy with an increasing number of incidences each year. The most frequently occurring genetic alterations detected are the ret/PTC rearrangements and BRAF mutations [3].
b) Cervical Cancer is the second most common cancer in women worldwide, with >90% of the cases being associated with an infection of a high-risk type of Human Papilloma virus (HPV16/HPV18).

MATERIALS AND METHODS

Material
Thyroid and cervical cancer cell lines [Table 1]
Normal cervical epithelium
Methods
RNA isolation from cell lines: mirVana™ miRNA Isolation Kit
Reverse Transcription: High Capacity Reverse Transcription Kit
Real-Time PCR: TaqMan® MicroRNA Assays on an Applied Biosystems 7900HT Fast Real-time PCR instrument

Thyroid Cell Lines	
N-thy-ori	Normal thyroid follicular epithelial cell line
N-thy-ret	Normal cell line, transfected with ret/PTC-1 mutation
TPC-1	Carcinoma cell line with Ret/PTC-1 mutation
N-thy-BRAF	Normal cell line, transfected with BRAF mutation
KAT10	Papillary thyroid carcinoma cell line with heterozygous BRAF mutation (V600E)

Cervical Cell Lines	
C33A	HPV negative
CaSki	HPV 16 (500 integrated copies) and HPV 18 positive

Table 1: Thyroid and cervical cancer cell lines

RESULTS

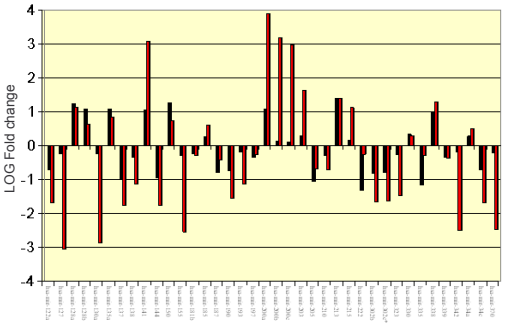

Fig. 1: Differentially expressed miRNAs in BRAF V600E harbouring cell lines versus normal thyroid cells.

Expression data were normalized to an endogenous miRNA control and to the normal thyroid cell line. The relative quantities are displayed as x-fold changes. In summary, fifteen miRNAs were up- and 23 miRNAs downregulated in both BRAF containing cell lines in comparison to the normal thyroid cell line [4].

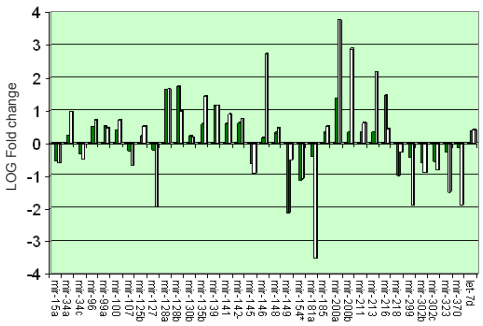

Fig. 2: Differentially expressed miRNAs in ret/PTC harbouring cell lines versus normal thyroid cells

Expression data were normalized to an endogenous control and to the normal thyroid cell line. The relative quantities are displayed as x-fold changes. In summary, twenty miRNAs were up- and 14 miRNAs downregulated in both ret/PTC containing cell lines in comparison to the normal thyroid cell line.

MiRNA	Potential Target mRNA
hsa-miR-200a	HIC2, RUNX1,MAP3K3, MAP2K4, PRKACB, RARB, DIO2
hsa-miR-200b	LAMC1,EIF4A2, CDK5R1, KLF3, MTSS1,PCDH7, TAF4, PTPN12
hsa-miR-141	STAT5A, THRAP2, DEK, RAB35,RAP2C, CTNND2, JUN, RAB38, TGFB2, GAP43, MMP11, DDX5, FGFR2, PRKACB
hsa-miR-127	CDC10, TNFSF12, RIMS4, MAPK4, TAF12,TFF1, SLIT1, APOB
hsa-miR-130a	LGR8, RXRA, TAF4, RAP2C, RAB30, ERBB4, FOSB, KIT, SPHK2, ELMO1
hsa-miR-144	PTHLH, MYCN, MET, PIM1, TIMP3, CDC42, MAP4K4

Table 2: Potential targets of miRNAs relevant in the context of Papillary Thyroid Carcinoma.

Three databases for target prediction have been used: MiRBase [5], PICTAR [6] and TARGETSCAN [7]. Analysis was performed for the most differentially expressed miRNAs. The putative targets have implications in thyroid carcinoma progression such as cell adhesion, MAPKKK cascade and oncogenesis.

miRNA	C33A HPV-	CaSki HPV+	Potential mRNA Targets
hsa-miR-10a	1.8 ↓	3.7 ↑	MYBL2, E2F3, BACH2 (Transcr regulator protein)
hsa-miR-23a	1.4	4.8 ↑	Zink finger protein ZFD25
hsa-miR-31	1.4	12 ↑	DNA-binding protein SATB2, BACH2, Proto-oncogene AF4
hsa-miR-34cN	12 ↓	5 ↑	Me-CP2 protein
hsa-miR-98	2 ↓	3.4 ↑	BACH1
hsa-miR-107	1.2	8.6 ↑	CHEK1, MTHFR, DICER1, BACH2, CDK6
hsa-miR-132	2.6	1.6 ↓	E2F5, MMP16, CDC40, FOSB, CyclinG1, CDKN1A
hsa-miR-135b	2.5 ↓	13.4 ↑	Note !
hsa-miR-137	1.4	12.4 ↑	Note !
hsa-miR-141N	200 ↓	17.3 ↑	Note !
hsa-miR-200cN	240 ↓	4 ↑	ARHE, USP25, DX
hsa-miR-324-3p	2.4 ↑	2 ↓	ORC6L
hsa-miR-324-5p	5 ↑	1	CDC14B, E2F3
hsa-miR-331	2.5 ↑	1.7 ↓	MYBL2, CDC6
hsa-miR-339	8.2 ↑	1	CDC14B
hsa-miR-345	11 ↑	1	Cyclin D2, IL4I, HDAC4
hsa-miR-373*	3.5 ↑	1	ZNF385, MARK4, Cytochrome C

Table 3: Differentially expressed miRNAs in HPV positive and negative cell lines normalized to normal cervical tissue

Expression data were normalized to an endogenous miRNA control and to normal cervical epithelium. The relative quantities are displayed as x-fold changes with upregulated miRNAs shown in red and downregulated miRNAs in green. The potential targets were predicted using miRanda Webserver & TargetScan.

Note 1) To date, these human miRNAs are not included in the miRanda database

CONCLUSIONS

Thyroid cancer cell lines: Specific miRNA patterns associated with different pathological pathways (ret/PTC and BRAF) were successfully identified.
Cervical cancer cell line: A distinctive miRNA expression signature which clearly distinguishes the infected cells from uninfected cells and normal cervical tissue was observed.

The potential targets of individual miRNAs were predicted using web-based algorithms. However, the identification of those targets that are biologically relevant for the disease or pathway under study remains a big challenge. Currently there does not exist an objective, un-biased method for this type of data interpretation.
The analysis and interpretation of the expression data was a bottleneck. Therefore, the authors strongly suggest the development of an appropriate software solution in order to facilitate the correlation of the miRNA expression data with both the predicted mRNA targets as well as the microarray expression data in a multidimensional analysis.

REFERENCES

1. Miska EA 2005 Curr Opin Genet Dev 15: 563-568
2. Esquela-Kerscher A, Slack FJ 2006 Nat Rev 6: 259-269
3. Sheils O 2005 Expert Rev. Mol. Diagn 5 (6): 927-946
4. Cahill et al 2006 Molecular Cancer 5:70
5. Griffiths-Jones S et al. 2006 NAR 34: 140-144
6. Krek A et al. 2005 Nat. Genet 37: 495-500
7. Lewis BP et al. 2003 Cell 115: 787-798