

Widespread Dysregulation of MiRNAs in Neuroblastoma: Association of MiRNA Expression with Survival

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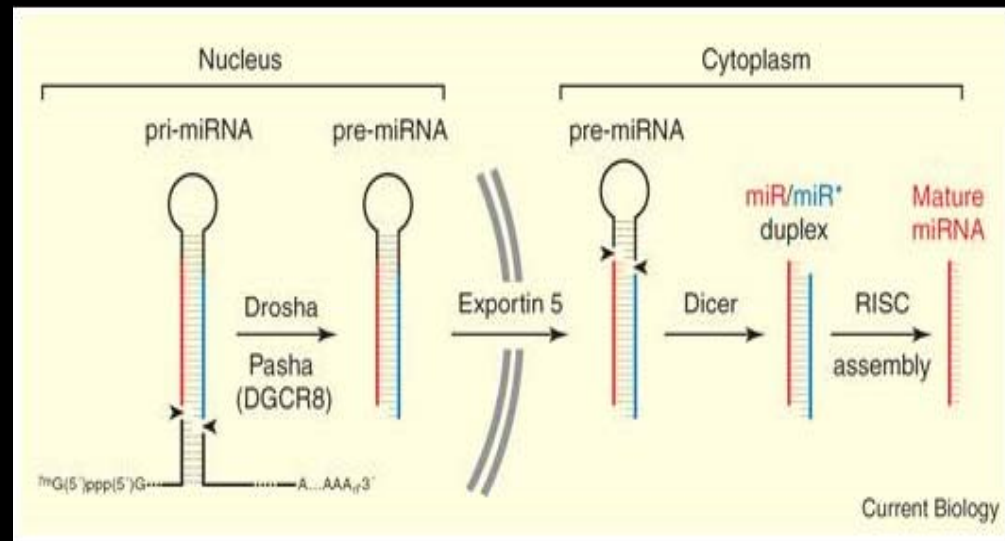


microRNA

- Mature microRNAs are single stranded RNAs ~22nt in length
- Function in the post-transcriptional regulation of gene expression predominantly through base-pairing with the 3'UTR of target mRNA causing

- mRNA cleavage and degradation
- Inhibition of protein translation

Biogenesis:



Seed region:

Bases 2- 8 at the 5' region of the mature microRNA.

Level of complementarity in this region will determine efficiency of binding to the target.

Impact of microRNA regulation

- Sanger miRBase Sequence Database - Release 16.0 (Sept. 2010) contains **1212** mature human miRNA
- Algorithm for the prediction of microRNA targets
 - *MicroCosm*
 - *TargetScan*
 - *PicTar*
- Each miRNA is predicted to have between 10's to 100's of mRNA targets

Why microRNAs as biomarkers?

- New approaches that can complement and improve cancer detection are needed.
- Several independent studies across different cancers
 - Significant differential expression of microRNA associated with cancer
- Assessment of microRNA expression is invaluable in the evaluation of human cancer

Advantages of looking at miRNA profiles

Durability:

miRNA~22nt in length -
detectable in even the most
highly degraded material.



Tissue



Serum

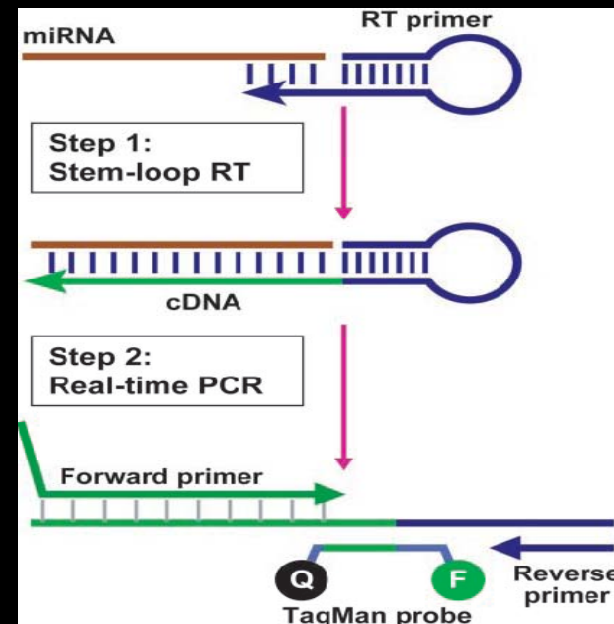


FFPE

microRNA
extraction &
profiling

Specificity:

Stem-loop RT PCR
bind to the 3' portion of mature
miRNA - RT product quantified
using conventional TaqMan PCR
(miRNA-specific primers)



Individual microRNA as common markers

- Certain microRNA already emerging as potential common markers
- E.g. miR-21
 - Up-regulated in cancer demonstrated in 10 independent studies
 - Breast, brain, lung, colorectal, pancreatic, prostate and cervical

microRNA signatures

- **Already established:**
 - microRNA expression signatures specific to cancer types & subtypes
 - Signatures predictive of survival
- microRNA expression in response to treatment is ongoing
- **Future:**
 - Signatures predictive of drug sensitivity and resistance will prove invaluable in the individual treatment of patients

microRNA survival signature in neuroblastoma

- One of the most frequent solid tumours in children, responsible for ~15% of all childhood cancer deaths.
- The clinical course of Neuroblastoma is extremely heterogeneous
 - Tumours with favourable biological features capable of spontaneous regression or differentiation into benign derivatives without therapy
 - Tumours with unfavourable biology that fatally progress despite advanced radiation and chemotherapy

MiRNAs Differentially Expressed in NBL Genetic Subtypes

Previous work: Chen & Stallings

157 microRNAs in 35 tumours.

Direct association of miRNA expression with patient survival could not be accomplished in such small data sets.

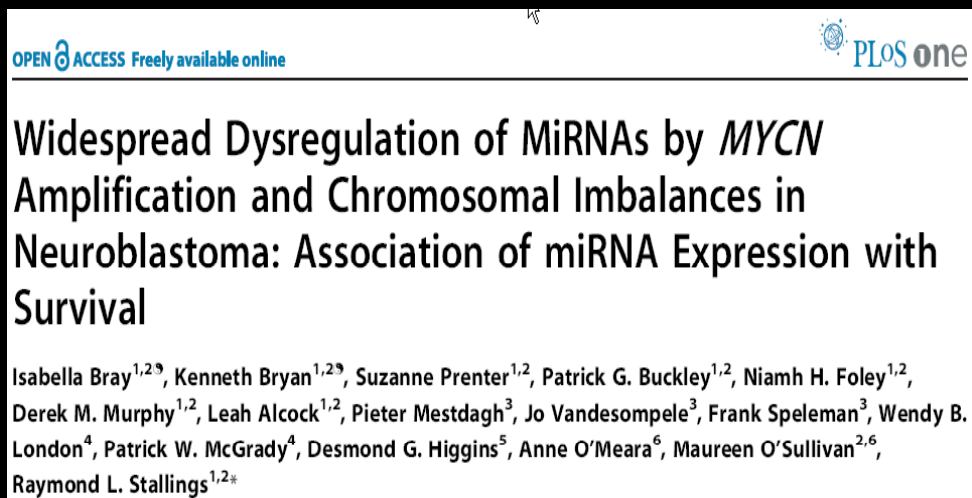
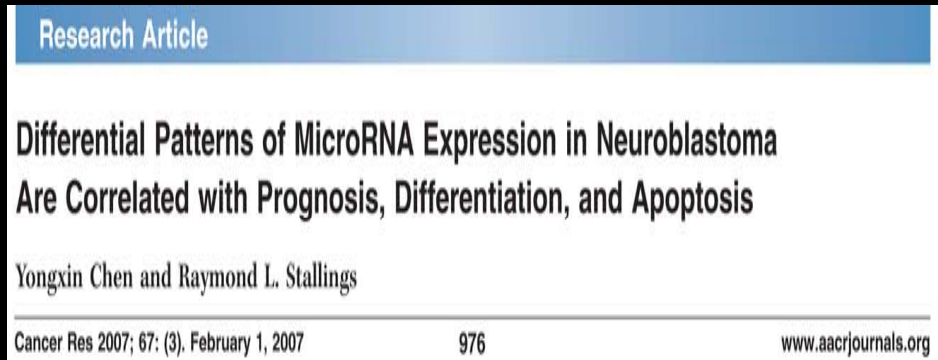
Latest work:

430 miRNA 145 tumors.

Also characterized by aCGH.

Identified miRNAs associated with

- patient survival
- genetic subtypes
 - over-expression of MYCN
 - large-scale chromosomal imbalances.



Patient cohort

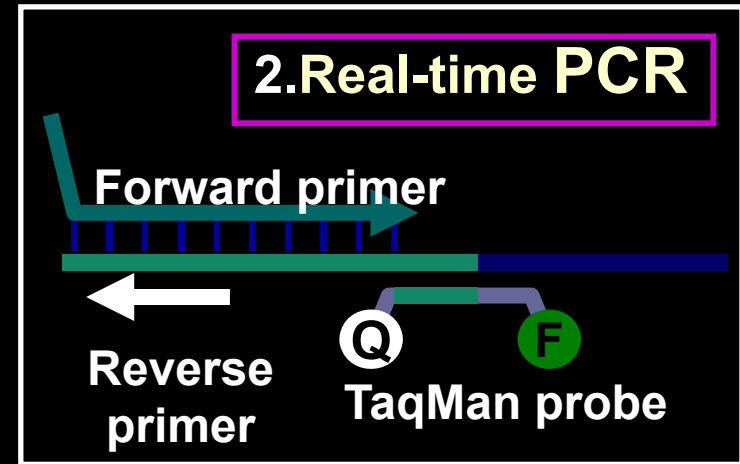
- Children's Oncology Group Philadelphia (n=108)
- Our Lady's Children's Hospital, Dublin (n=37).
- Patients were treated under either the U.S. neuroblastoma treatment protocol or the European treatment protocol between 1998 to 2004.
 - stage 4 (n=74)
 - stage 3 (n=31)
 - stages 1, 2 and 4s (n=40)
- 36 - MYCN amplification
- 42 - loss of 11q without MNA.
 - **Tumors with amplification of the *MYCN* transcription factor represent a major genetic subtype of metastatic neuroblastoma with a particularly poor prognosis, as do those with hemizygous loss of a large segment on chromosome 11q.**

Analysis of samples using RTqPCR

1. **RNA-extraction** preserves small RNAs (Qiagen miRneasy®)

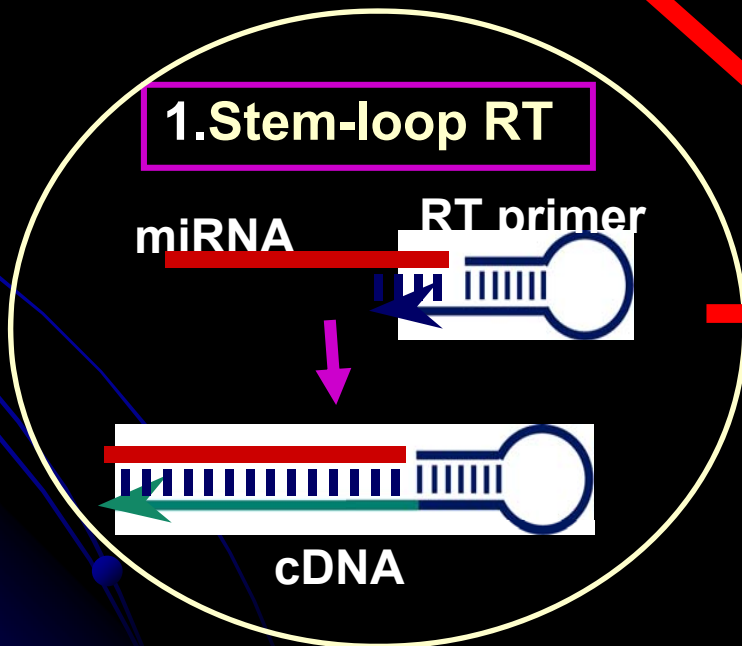


4. **qPCR**
Manually spotted 384-well plates



2. **Reverse Transcription**

Megaplex
TaqMan®
MicroRNA
Assays
simultaneous RT
of 450 mature
miRNAs



3. **cDNA pre amplification** reduces the amount of input RNA to 20ng per sample

Analysis of 430 miRNA across 145 tumour samples

- For each sample 430 miRNA were profiled using a real-time quantitative PCR platform.
 - 132 miRNA expressed in <10 samples
 - Final data set = 298 miRNAs in at least 10 samples
- We split the data into a *training set* (66%) and a *validation set* (33%).
- The validation set played no part in training or testing and was used to independently validate our findings.
- The selection was stratified in that approximately the same rate of deaths occurred in both sets.

A miRNA expression profile associated with MYCN amplified tumours

17-5p-92

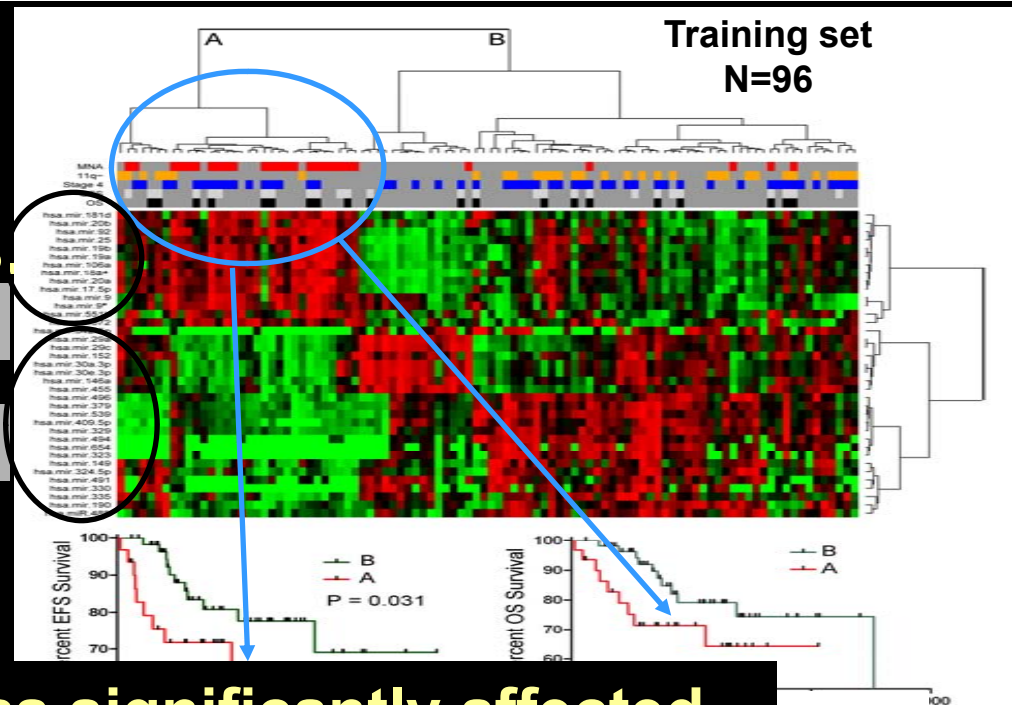
379-656

Significance of miRNA differentially expression

- p value assigned
- Corrected for multiple

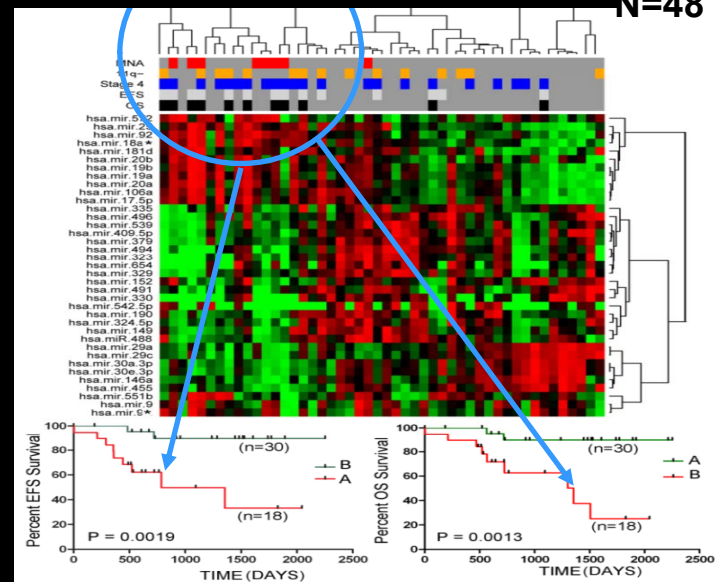
37 differentially expressed ($p < 0.05$).

Hierarchical cluster analysis showed a significant split in MNA versus non-MNA in training and validation set.



MYCN amplification has significantly affected miRNA expression in neuroblastoma tumours

Validation set N=48



Identification of a miRNA expression signature predictive of clinical outcome in neuroblastoma

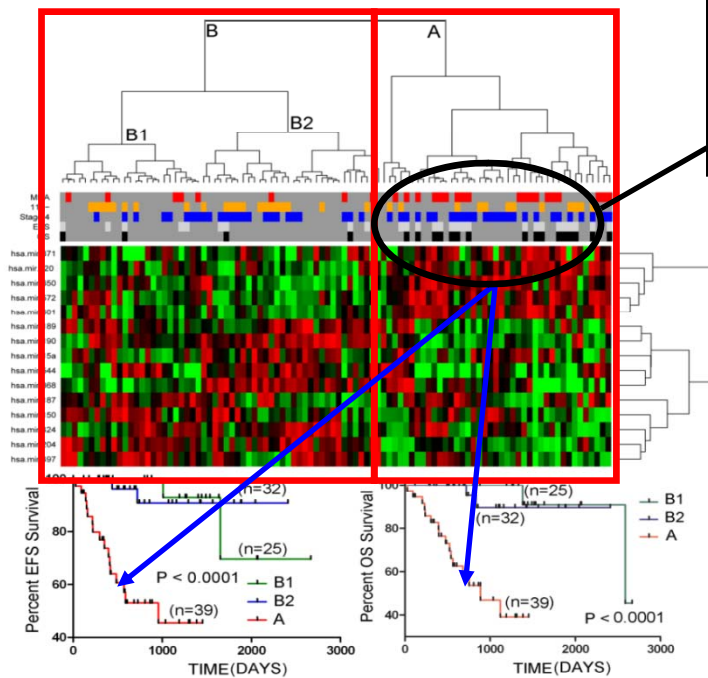
- Derived a signature predictive of outcome by identifying miRNA specifically associated with survival.
- Random Forest classifier method was used to identify miRNA most significantly associated with survival.
 - systematically removes miRNA
 - classifier is re-run.
 - large reduction in accuracy = Important miRNA
- Identified 15 miRNA most significantly associated with overall survival.

miRNA significantly associated with survival
Hsa-miR-520
Hsa-miR-371
Hsa-miR-572 *
Hsa-miR-650
Hsa-miR-368
Hsa-miR-15a
Hsa-miR-544
Hsa-miR-190 *
Hsa-miR-189
Hsa-miR- 424
Hsa-miR-204
Hsa-miR- 497
Hsa-miR- 187
Hsa-miR- 150
Hsa-miR- 601

* Also associated with MNA

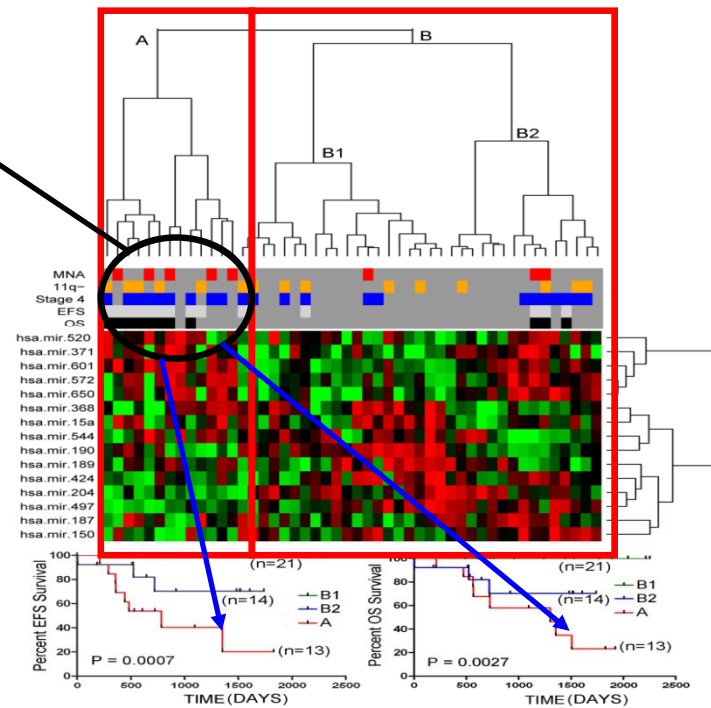
Result: miRNA expression signature predictive of clinical outcome in neuroblastoma.

- Hierarchical clustering using these miRNA produced two major clusters that differed significantly in survival



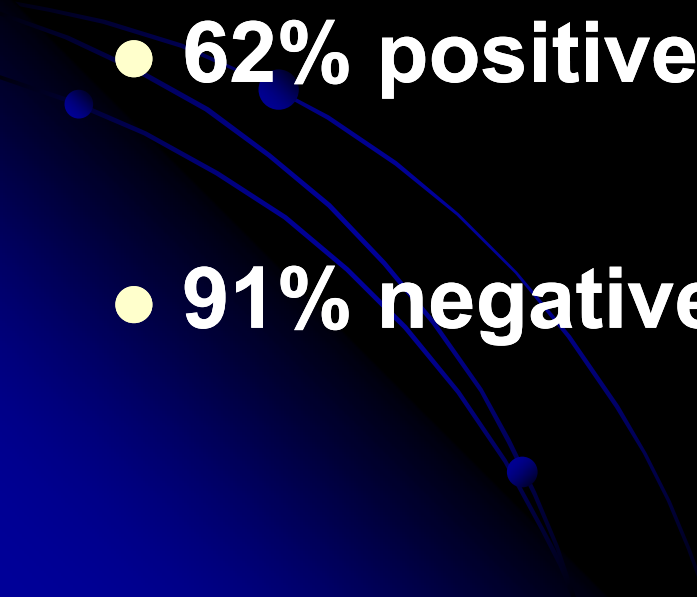
Training set

Stage 4
MNA
11q loss



Validation set

Specificity of miRNA survival signature

- 73% sensitivity
 - 87% specificity
 - 62% positive predictive value
 - 91% negative predictive value
- 

Stratification of 11q- tumors based on the miRNA survival signature.

Journal of Clinical Oncology, Vol 27, No 7 (March 1), 2009: pp. 1026-1033
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Pediatric Oncology

Overall Genomic Pattern Is a Predictor of Outcome in Neuroblastoma

Isabelle Janoueix-Lerosey, Gudrun Schleiermacher, Evi Michels, Véronique Mosseri, Agnès Ribeiro,

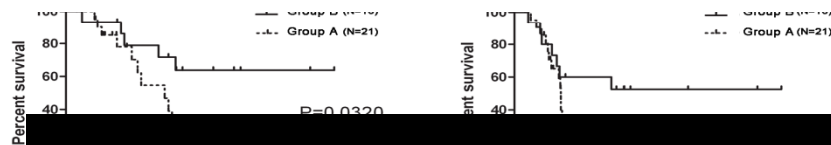
Oncogene (2010) 29, 865-875
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www.nature.com/onc

ORIGINAL ARTICLE

Human Cancer Biology

Chromosomal and MicroRNA Expression Patterns Reveal Biologically Distinct Subgroups of 11q- Neuroblastoma

Patrick G. Buckley^{1,2}, Leah Alcock^{1,2}, Kenneth Bryan^{1,2}, Isabella Bray^{1,2}, Johannes H. Schulte³, Alexander Schramm³, Angelika Eggert³, Pieter Mestdagh⁴, Katleen De Preter⁴, Jo Vandesompele⁴, Frank Speleman⁴, and Raymond L. Stallings^{1,2}



Clinical
Cancer
Research

ent set of tumours obtained from
University Hospital and University
Hospital, Essen.

ression signature divides
into groups with
different clinical outcome.

the number of segmental
imbalances.

with recent reports
overall pattern of genomic imbalances
is highly predictive of clinical outcome
in neuroblastoma

Potential for more refined patient stratification prior to therapy.

Do miRNA from the survival expression signature correlate with mRNA expression signatures of neuroblastoma?

	Upregulated miRNAs							
	hsa-miR-520f	hsa-miR-15a	hsa-miR-150	hsa-miR-572	hsa-miR-371-5p	hsa-miR-601	hsa-miR-187	hsa-miR-650
CADM1
CHD5
CLSTN1	.	0.0394
DDC	.	.	.	0.0019	.	.	0.0371	.
EPHA5
FYN	0.0096
HIVEP2
MAP7	0.0435	.
NRCAM
PDE4DIP	.	< 0.0001	0.0106
PIK3R1	.	.	.	0.0256
PLAGL1	0.0368
PLAT
PI
PI
PI
PI
PI
PI
U
WSD1	.	.	.	0.0121

We show an enrichment for predicted target sites in 3'UTR of previously published mRNA survival signatures.

Oberthuer *et al.*, 2006
 approaching significance
 p= 0.06
 Vermeulen *et al.*, 2009
 P=0.0007

miRNA signatures potentially regulate the gene survival signature.

Conclusion

- Our work identifies an miRNA signature predictive of poor survival, and associated with many biological features of this disease.
- We show a miRNA expression signature, combined with analysis of segmental imbalances, provides greater prediction of survival outcome than 11q status alone.
- Both potentially improving patient stratification before therapy.
- **Future Work**
- These miRNAs are being prioritized for further investigate into their involvement in disease pathogenesis.



Cancer Genetics:

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Dr. Jackie Ryan

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