microRNA Signatures in Cancer

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microRNAs: The Basics

- Discovered in 1993: central to cell differentiation & development in *C. elegans*
  - microRNAs are ~22nt long single stranded RNAs
- Post-transcriptional regulation “Master control” at translation of protein coding genes
- Identified in humans in 2001 with ~2000 found since
  - Linked to diseases, notably cancer, immune mediated disorders, viral infections, cardiovascular disease, neurological and metabolic disorders

Winter et al., Nature Cell Biology 2009
Biologic role makes microRNAs ideal cancer biomarkers & potential therapeutic candidates

- MicroRNAs are aberrantly expressed in all cancers studied
- MicroRNAs are oncogenes and tumor suppressors
- microRNAs target self sufficiency in growth signals, apoptosis evasion, limitless proliferation, sustained angiogenesis and tissue invasion and metastasis
- MicroRNA genomic deletions correlate with specific cancers (B-cell lymphoma)
MicroRNAs are ideal biomarkers for cancer diagnostics

- Short RNAs embedded in protein complex: robust to degradation
- Stable markers in a variety of tissue samples and body fluids
- Stable at room temperature for years under FFPE conditions
- MicroRNAs identify tissues by source regardless of sample preparation
- Expression profiles classify most tumors with high accuracy and may predict clinical outcomes
MicroRNAs identify tissues by source regardless of sample preparation

- microRNA expression profiles cluster according to the patient/source whether sourced as fresh-frozen or formalin-fixed paraffin-embedded (FFPE) tissues;
- However, mRNA expression profiles most closely reflect the sample type

MicroRNAs demonstrate high tissue specificity

- Epithelial origin separated from non-epithelial origin using only 2 microRNAs
  - miR-200c and miR-205
- Published by Rosetta in March 2008

- Subsequent independent papers suggested same microRNAs control epithelial mesenchymal transition (EMT)

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Historically, in ~20%-30% of cases, specimen quantity & quality, and available methodology limits correct classification of lung tumors.

- qRT-PCR microRNA analysis of 8 miRs clearly distinguishes between the 4 main subtypes of lung tumors.

- Highly suitable for sub-optimal Fine Needle Aspirates (FNA), brushings, and other small specimens.

High tissue specificity translates into an accurate lung cancer assay using only small tumor samples.
Lung cancer discrimination validated performance higher than histopathology

<table>
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<th>Squamous</th>
<th>Non-Squamous</th>
<th>Carcinoid</th>
<th>Small Cell</th>
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<td>153</td>
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<tr>
<td>In-correctly classified</td>
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<td>13</td>
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<tr>
<td>Total</td>
<td>139</td>
<td>166</td>
<td>35</td>
<td>70</td>
<td>41</td>
<td>451</td>
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</tbody>
</table>

- Overall Sensitivity: = 93.7%
- Overall Specificity: = 97.9%
  - Sensitivity for pathology specimen: = 92.1%
  - Sensitivity for cytology specimen: = 95%
- Overall success rate (samples with answer): = 91%
- Overall correct samples: 384/410

Similarly, high tissue specificity translates into an accurate renal cancer subtyping assay

Renal tumors assessed by KNN (5) algorithm using 24 microRNAs

Overall 174/184 classified correctly → 95% accuracy

1 sample failed sample QA due to insufficient RNA;
16 samples: no results were generated (8%)
microRNA based decision tree also sorts multiple cancer types effectively
Microarray analysis of 64 miRNAs can identify 42 distinct tumor origins

- Trained with ~1300 FFPE tumor samples
- Independent validation test set of 509 samples
  - 146 (30%) were metastatic tumors
  - Result of 1 or 2 tumor types generated from two algorithms
  - Only 20 samples (4%) failed to produce result
- Overall, the correct tissue of origin was predicted by at least one of the reported answers in 418 (85%) (“sensitivity of prediction”)
  - 403 samples (82%) received a single answer, of which 361 (90%) were classified correctly
  - Overall specificity up to 99%
  - 54 samples (11%) resulted in a “unified categorical” answer, e.g. Sarcoma, RCC, Thyroid type not specified, etc. (7-categories)

Most robust discrimination achieved via two discrete complementary classifiers

Two classifiers are employed to interpret the expression of 64 microRNAs as a combined classifier.
Studies in actual CUP patients at leading international centers show 80-92% concordance

- Prospective study on 75 CUP patients at M.D. Anderson done on first generation assay (narrower range of miRNAs & tumor types) and results corroborated with second generation assay
  - **84% concordance** with final diagnosis\(^1\)
- Two studies (first* & second** generation) on 52 real CUP patients as well as on metastases of known origin at Heidelberg University
  - **80%* & 88%**\(^{**}\) concordance with final diagnosis\(^2,3\)
- Study on 84 real CUP patients at University of Ioannina & Hellenic Cooperative Oncology Group
  - **92% concordance** with final diagnosis\(^4\)

Retrospective cohort of resected metastatic lesions from 84 CUP patient\(^1\)

Each prediction was assessed for agreement with:
- Pathological information
- Clinical information
- Follow-up and outcome data

92% concordance overall

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Potential impact of microRNA test results in Ioannina University Hospital CUP cases

- In 77 patients (92%) test results were fully concordant with final diagnosis

- For 18 patients (21%), following test results would have resulted in administration of different chemotherapeutic regimens
  - In 9 patients, different combination chemotherapies likely to be more active possibly with superior survival
  - In 16 patients, the change in diagnosis could have been coupled to a change in targeted therapy employed

- In the remaining patients, results in agreement with the presenting diagnosis were still deemed useful objective information to narrow the differential diagnosis and increase the confidence level for treatment strategy

Case report—brain metastases in a woman with triple negative breast cancer (TNBC)

Clinical presentation:
Female presented with breast mass several months earlier

Pathology: Dx TNBC
- Poorly-differentiated adenocarcinoma with tumor giant cells, adenoid formations
- IHC: negative for Estrogen-Receptor, Progesterone-Receptor and Her2/neu
Case report- triple negative breast cancer in actuality CUP from “lost” melanoma

- microRNA microarray test result: **Melanoma**
- IHC after molecular profiling consistent with microRNA:
  - HMB45 - positive
  - S-100 - focally positive
  - MELAN A – negative
- Breast lesion was then molecularly profiled on microRNA array and resulted in the same diagnosis of melanoma
- IHC for breast lesion same as metastasis

Published in: The Oncologist 16:165-74, 2011-”Accurate classification of metastatic brain tumors using a novel microRNA-based test” Muller, Spector et al.
The challenge of triple negative breast cancer (TNBC): can microRNA open new paths?

- In TNBC cells, 13 microRNAs recently reported as dysregulated versus normal\(^1\)
- Metastatic signature of TNBC: 6 microRNAs differentially expressed in tumor vs. metastasis and in normal vs. metastasis, but not in normal vs. tumor
- miR-424 is down regulated in mets vs. primary
  - Aberrant miR-424 has been observed in progression of other cancer types, and may indicate a role in the development of metastases\(^2\)

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microRNA expression signatures may be able to stratify survival in TNBC

- 4 microRNAs significantly associated with overall survival of TNBC patients
- Combined 4-miR signature stratified median overall survival of 69 vs. 83 month between the high and low microRNA-based risk groups

Ovarian Cancer Response To First Line Platinum Based Treatment

- microRNAs from FFPE tumor samples of 57 ovarian cancer patients receiving platinum-based therapy as first line hybridized to Rosetta’s microarrays
- 7 microRNAs were found to be significantly differentially expressed in tumors from platinum sensitive vs. platinum resistant patients
- 5 microRNAs were associated with significant difference in survival or recurrence-free survival

High expression of hsa-mir-27a identified a sub-group with very poor prognosis

Mesothelioma diagnosis: orphan clinical unmet need of increasing importance

- 61-year-old female presented with peritoneal carcinomatosis and ascites
- Presumptive diagnosis: primary peritoneal/ovarian carcinoma.
- Failed response to taxane-platinum therapy, but had indolent course, with overall survival of 30 months
- Later microRNA profile gave a single answer: mesothelioma
  - Additional IHC tests confirmed diagnosis
- This guided diagnosis might suggest alternative therapy of pemetrexed/platinum

Results of further IHC performed following microRNA test results:
A H&E  B CK5/6
C Calretinin  D Mesothelin

G Pentheroudakis, N Pavlidis et al. A Novel microRNA-based Assay demonstrates 92% accuracy in classification of metastatic tumors from patients diagnosed with carcinoma of unknown primary. Poster presentation at ASCO meeting in Chicago, June 2012
Mesothelioma prognosis: orphan clinical unmet need of increasing importance

- Despite indolent course in previous patient, mesothelioma remains highly lethal with Median TTP and survival after surgery 8-12m and 12-20m
- Currently, inability to forecast outcomes limits clinicians from directing aggressive therapy to the individuals who may actually benefit from such an approach
- Elevated miR-29c* Associated With Better Prognosis

Published Online First on February 16, 2010 as 10.1158/0008-5472.CAN-09-3993

Molecular and Cellular Pathobiology

hsa-miR-29c* Is Linked to the Prognosis of Malignant Pleural Mesothelioma

Harvey I. Pass1, Chandra Goparaju1, Sergey Ivanov1, Jessica Donington1, Michele Carbone2, Moshe Hoshen3, Dalia Cohen3, Ayelet Chaulet3, Shai Rosenwald3, Harel Dan3, Sima Benjamin3, and Ranit Aharonov3
Functional Consequences of miR-29c* Overexpression in Mesothelioma Cell Lines

Mir29c* is higher in normal mesothelial cell lines compared with MPM
Associated with a more differentiated phenotype
Functional analyses data is compatible with the clinical findings

Pass HI, Goparaju C, Ivanov S et al. hsa-miR-29c* is linked to the prognosis of malignant pleural mesothelioma. Cancer Res. 2010 Mar 1;70(5):1916-24
Elevated miR-29c* Associated With Better Mesothelioma Prognosis

- miR-29c* had significantly different expression (p = 0.00036, FC 1.8) between long and short TTP
- Elevated miR-29c* was associated with longer survival time in both training and test sets

Pass HI, Goparaju C, Ivanov S et al. hsa-miR-29c* is linked to the prognosis of malignant pleural mesothelioma. Cancer Res. 2010 Mar 1;70(5):1916-24
miRNA biomarkers for stratification of response to novel immunotherapy

- Experimental immunotherapy trial in metastatic solid tumor
- 12 miRNAs identified differentiating 10 responders from 10 non-responders at Day30 (all passed FDR P<0.05)
- A classifier using two miRNA’s from day 30 markers also shows good signal at outset of therapy
Looking Forward with microRNAs

- **Blood-based diagnostics**

- **Additional Response/Predictive Biomarkers for New Drugs**
  - Identify patient sub-cohorts that respond well to drugs stalled in development
  - Response prediction suggested in several cancer types to date

- **Therapeutics**
  - Up or down regulating a microRNAs can regulate entire pathway
  - Liver cancer (HCC): animal POC successfully completed & published
  - Ovarian cancer: initial, pre-clinical phase
  - Recent studies in triple negative breast cancers suggest opportunity

- **Non-oncology applications**