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Abstract: Malignant pleural mesothelioma (MPM) is a tumor originating in the mesothelium, the membrane lining the thoracic cavities, and is induced by exposure to asbestos. Australia suffers one of the world’s highest rates of MPM and the incidence is yet to peak. The prognosis for patients with MPM is poor and median survival following diagnosis is 4-18 months. Currently, no or few effective therapies exist for MPM. Trials of targeted agents such as antiangiogenic agents (VEGF, EGFR) or ribonuclease inhibitors (ranpirnase) largely failed to show efficacy in MPM Tsao et al. (2009) [1]. A recent study, however, showed that cisplatin/pemetrexed + bevacizumab (a recombinant humanized monoclonal antibody that inhibit VEGF) treatment has a survival benefit of 2.7 months Zalcman et al. (2016) [2]. It remains to be seen if this targeted therapy will be accepted as a new standard for MPM. Thus the unmet needs of MPM patients remain very pronounced and almost every patient will be confronted with drug resistance and recurrence of disease. We have identified unique gene signatures associated with prolonged survival in mesothelioma patients undergoing radical surgery (EPP, extrapleural pneumonectomy), as well as patients who underwent palliative surgery (pleurectomy/decortication). In addition to data published in Molecular Oncology, 2015;9:715-26 (GSE59180) Kirschner et al. (2015), we describe here additional data using a system-based approach that support our previous observations. This data provides a resource to further explore microRNA dynamics in MPM.

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Data in Brief

MicroRNA gene expression signatures in long-surviving malignant pleural mesothelioma patients

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ABSTRACT

Malignant pleural mesothelioma (MPM) is a tumor originating in the mesothelium, the membrane lining the thoracic cavities, and is induced by exposure to asbestos. Australia suffers one of the world’s highest rates of MPM and the incidence is yet to peak. The prognosis for patients with MPM is poor and median survival following diagnosis is 4–18 months. Currently, no or few effective therapies exist for MPM. Trials of targeted agents such as antiangiogenic agents (VEGF, EGFR) or ribonuclease inhibitors (ranpirnase) largely failed to show efficacy in MPM Tsao et al. (2009) [1]. A recent study, however, showed that cisplatin/pemetrexed + bevacizumab (a recombinant humanized monoclonal antibody that inhibit VEGF) treatment has a survival benefit of 2.7 months Zalcman et al. (2016) [2]. It remains to be seen if this targeted therapy will be accepted as a new standard for MPM. Thus the unmet needs of MPM patients remain very pronounced and almost every patient will be confronted with drug resistance and recurrence of disease. We have identified unique gene signatures associated with prolonged survival in mesothelioma patients undergoing radical surgery (EPP, extrapleural pneumonectomy), as well as patients who underwent palliative surgery (pleurectomy/decortication). In addition to data published in Molecular Oncology, 2015;9:715-26 (GSE59180) Kirschner et al. (2015) , we describe here additional data using a system-based approach that support our previous observations. This data provides a resource to further explore microRNA dynamics in MPM.

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Keywords:
- microRNA
- Mesothelioma
- Pathway
- Systems biology
- Therapeutic agents

Sample source location
Sydney, Australia

1. Direct link to deposited data

MicroRNA profiling of malignant pleural mesothelioma tumour tissues, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59180

2. Experimental design, materials and methods

The experimental design and analysis pipeline are outlined in Fig. 1. Briefly, we used 16 FFPE (formalin-fixed paraffin embedded) tumor samples from patients who underwent EPP (extrapleural...
pneumonectomy) [3,5] to interrogate the prognostic value of genome-wide microRNA gene expression. The RNA samples were divided into 2 groups; 1) long survival, median = 53.7 months, n = 8 and 2) short survival, median = 6.4 months, n = 8. Agilent Human 8x15k miRNA microarrays (GPL10850) were utilized for microRNA transcriptome profiling. Agilent feature extraction v10.5 was used to extract fluorescence intensity.

2.1. Data processing

Total Gene Signals from the miRNA arrays were quantile normalized and log transformed to base 2. Further filtering was applied to exclude ones with gene expression of < 1 (Partek Genome Suite, v6.6). In comparison, Kirschner et al., 2015 used signal value of 1 as a threshold and normalized by shifting to the 90th percentile without applying baseline transformation. The intention here is to show that regardless of normalization methods used for the initial array data processing (Fig. 1), candidate microRNAs identified still hold true their association with the phenotype and in particular, the affected regulatory pathways where predicted genes are targeted by these microRNAs.

One-way ANOVA was used to examine differential gene expression between long and short survival (n = 16 in total). This analysis also assumes that long and short survival-related gene expression patterns are normally distributed and that the variance is approximately equal between the groups. Configuring the ANOVA in Partek Genome Suite (v6.6) enabled adjustment for systematic technical errors, such as batch processing of the RNA samples as well as the shortcomings of RNA extraction from FFPE blocks.

2.2. Visualization of gene expression and regulatory pathways

To visualize the dataset, further analysis was carried out to identify distinctive gene expression patterns using Self-Organizing Map (SOM) clustering (map height = 4, map width = 3) (Fig. 2A). Cluster structure of this dataset in the form of non-linear mapping to a two-dimensional grid (SOM clustering) enabled us to explore the relationship and ultimately the function of these microRNAs. Hierarchical clustering (Fig. 2B) was also utilized (subdivide gene expression in similar vs different clusters) to identify significantly enriched functional categories. This is useful for unknown genes in the same cluster to enable us to infer a functional role. Two distinctive SOM clusters were identified and assigned to “Long Survival” and “Short Survival” based on the following criteria; 1) P < 0.05 differentially expressed, 2) Fold change > 1.5 and 3) correlation with enriched pathway based on predicted targets. We focused on 2 clusters, cluster 1 and cluster 11, as they showed distinct expression differences between long and short survival (Fig. 2A, Table 1).

Predicted target genes of microRNAs in the “Long Survival” and “Short Survival” clusters were extracted from miRDB [6] (Version 6.0, release date: August 2014) and starBase Version 2.0 [7]. Within starBase, predicted targets were extracted based on intersections with TargetScan [8], picTar [9], RNA22 [10], PITA [11] and miRandA/miSRV [12]. This resolves some of the issues surrounding use of predicted targets from bioinformatics approaches where validation of targets leads to an estimated false positive rate of at least 20–40% [13,14] and false negative rates of up to 50% [15,16]. Although protocols such as HITS-CLIP [17] or PAR-CLIP [18] are considered in starBase for determining RISC occupancy on microRNAs, similar pitfalls still exist and we proceed with caution. The parameters used for starBase are medium stringency (≥2CLIP-Seq experiments supported the predicted miRNA target site).

To interrogate the data further, gene ontology (GO) enrichment analysis based on these target gene lists (Fisher’s Exact test) was carried out to detect over-represented genes and to reflect enriched biological themes (biological process, molecular function and cellular component) as described [3]. Pathway enrichment analysis was carried out on this predicted target gene list to explore association of enriched regulatory pathways with patient survival outcome. This is based on Fisher’s Exact test (P < 0.05), using the KEGG (Kyoto Encyclopedia of Genes and Genomes) [19] Homo sapiens hg19 Build reference genome as the background [3] as well as hg38 Build (Fig. 1). The aim was to
A. SOM Clustering

![Fig. 2. Visualization of microRNA microarray data. (A) SOM clustering of distinctive microRNA gene expression patterns in long vs short survival patients. (B) Hierarchical clustering showed distinctive gene expression pattern of candidate microRNAs to reflect coordinated regulation in long vs short survival patients.](image)

Table 1

| Long survival microRNAs vs short survival microRNAs by SOM clustering analysis. |
|---|---|---|---|---|
| Candidates microRNAs from [3]| |
| **Cluster 1: short survival microRNA** | p-Value (short vs long) | Fold-change (short vs long) | Fold-change (short vs long) | (description) |
| hsa-miR-210-3p | 0.0010555 | 4.02696 | Short up vs long | x |
| hsa-miR-93-5p | 0.0011103 | 3.3278 | Short up vs long | x |
| hsa-miR-221-3p | 0.0029257 | 6.45867 | Short up vs long | x |
| hsa-miR-22-3p | 0.0094396 | 2.2148 | Short up vs long | x |
| hsa-miR-151-5p | 0.0102997 | 2.39401 | Short up vs long | x |
| hsa-miR-20a-5p | 0.0102997 | 2.39401 | Short up vs long | x |
| hsa-miR-92a-3p | 0.0141019 | 1.69546 | Short up vs long | x |
| hsa-miR-30e-5p | 0.0196756 | 3.32221 | Short up vs long | x |
| hsa-miR-146b-5p | 0.021048 | 2.52768 | Short up vs long | x |
| hsa-miR-17-5p | 0.0219565 | 3.21235 | Short up vs long | x |
| **Cluster 11: long survival microRNA** | p-Value (short vs long) | Fold-change (short vs long) | Fold-change (short vs long) | (description) |
| hsa-miR-671-5p | 0.0012239 | 2.28734 | Short down vs long | x |
| hsa-miR-188-5p | 0.0036218 | 1.97211 | Short down vs long | x |
| hsa-miR-1469 | 0.0042418 | 3.8781 | Short down vs long | x |
| hsa-miR-654-5p | 0.0046082 | 12.2823 | Short down vs long | x |
| hsa-miR-622 | 0.0050860 | 1.974 | Short down vs long | x |
| hsa-miR-662 | 0.0067508 | 7.14828 | Short down vs long | x |
| hsa-miR-1471 | 0.0068843 | 3.04317 | Short down vs long | x |
| hsa-miR-1183 | 0.0083105 | 1.9464 | Short down vs long | x |
| hsa-miR-431-5p | 0.0100513 | 5.19376 | Short down vs long | x |
| hsa-miR-370-3p | 0.0111777 | 2.9025 | Short down vs long | x |
| hsa-miR-345-5p | 0.0122815 | 6.13592 | Short down vs long | x |
| hsa-miR-483-5p | 0.012858 | 1.80356 | Short down vs long | x |
| hsa-miR-877-3p | 0.0239915 | 1.66464 | Short down vs long | x |
| hsa-miR-1225-5p | 0.025947 | 1.56253 | Short down vs long | x |
| hsa-miR-30c-1-3p | 0.0262453 | 4.46393 | Short down vs long | x |
characterize and compare the biological pathways best represented in both series. The higher the enrichment score the more enriched the genes are within a specific pathway (Table 2).

As shown from alternate data processing (Fig. 1) and discovery processes (Fig. 2), the results from these additional analyses support the robustness of miR-Score outlined in Kirschner et al. [3]. In the first instance, the directionality of fold change of the candidate microRNAs agrees (Table 1) where gene expression of hsa-miR-21-5p, hsa-miR-221-3p and members of the hsa-miR-17-92 cluster (hsa-miR-17-5p, hsa-miR-20a-5p) were higher in short survivors [3]. Furthermore, these microRNAs have been shown by others to be involved in PTEN regulation and modulate the PI3K/Akt signaling pathway [20,21]. For visualization purposes, we compared genes targeted by these candidate microRNAs from pathways that have been found to be associated with cancer progression, tumor architecture and drug resistance [22,23], i.e., PI3K/Akt signaling, hippo signaling and focal adhesion pathways (Fig. 3, Table 3). All of which have been implicated in mesothelioma biology [24].

2.3. Utilizing microRNA-based regulation to exert a systems effect

Of note, visualizing expression of microRNA and its targets within a pathway and identifying commonalities between these pathways (Fig. 3) enable further exploration, especially in terms of designing microRNA therapeutic targets [25]. Complex disease conditions such as heart failure and cancer are increasingly being recognized as the result of multiple dysfunctional pathways. Hence it is essential to change our approach towards disease treatment and develop new therapeutic strategies that seek to correct the overall network of pathways that has been distorted. At a systems level, the aim of either rescuing defective genes within a beneficial pathway and/or shutting down pathological gene(s) upstream of a regulatory cascade becomes clearer using this type of visualization approach [26-28]. For example, CCND2, a target of hsa-miR-17-5p, appears to be common to the three enriched pathways mentioned above. Inhibiting CCND2 expression via hsa-miR-17-5p would thus modulate all three pathways and would have the potential to reduce tumor growth. According to starBase [7], CCND2 is also a target of hsa-miR-15/16 and thus can potentially be targeted by TargomiRs, a novel microRNA-based treatment approach for MPM using microRNA-loaded EGFR antibody-targeted minicells (EDV™nanocells) [29]. In KEGG [19], CCND2 is shown to be deeply entrenched in cell cycle progression, anti-apoptosis and pro-proliferation processes. Furthermore, in the case where gene expression of hsa-miR-21-5p, hsa-miR-221-3p, hsa-miR-17-5p and hsa-miR-20a-5p is higher in short survivors, an anti-microRNA approach [27] would be appropriate to restore the suppressed beneficial pathways.

In contrast, where down-regulated microRNAs are associated with a disease condition, a re-introduction of these microRNAs can be utilized to exert a systems effect in order to ameliorate dysregulated pathways. For example, in MPM, miR-16, miR-15a and miR-15b mimics were observed to have tumor suppressing properties [30] and subsequently a mimic based on the consensus sequence of the miR-15 family has shown signs of activity in a phase 1 clinical trial in MPM patients [29]. The assumption here is that detrimental pathways were rescued genomewide. In comparison, a common seed-like sequence designed to exert microRNA-like regulation at 3’UTR region of unrelated genes [31,32] can also have a systems effect.

Using the Molecular Signatures Database (MSigDB, now v5.1 [33]) to apply Gene Set Enrichment Analysis (GSEA) to differentially expressed genes from four MPM gene expression datasets, enabled us to identify enriched microRNA binding motifs that can be translated into potential druggable targets for MPM [34]. Here, GSEA on the twenty genes (Table 3) extracted from the enriched pathways revealed promoter regions (~2 kb, 2 kb) around transcription start sites containing enriched binding motifs for transcriptional factors; MAZ (GGAGGR), PAX4 (GGTGGR), AP1 (TGANTCA) and FOXO4 (TGTGTT). All of which are implicated in MPM biology [35-37]. Using ChIPBase database (curated

### Table 2

| Pathway name                      | Enrichment score | Enrichment p-Value | # genes in list, in pathway | Pathway ID          |
|-----------------------------------|------------------|--------------------|-----------------------------|---------------------|
| Vasopressin-regulated water reabsorption | 7.88895          | 0.000374863        | 5                           | kegg_pathway_57     |
| Hippo signaling pathway           | 6.25988          | 0.00191147         | 8                           | kegg_pathway_96     |
| Pancreatic cancer                 | 5.794            | 0.00304576         | 6                           | kegg_pathway_249    |
| Vascular smooth muscle contraction | 5.71464          | 0.00329734         | 7                           | kegg_pathway_118    |
| Chronic myeloid leukemia          | 5.57127          | 0.00380954         | 6                           | kegg_pathway_69     |
| Hepatitis B                       | 5.50279          | 0.00407539         | 8                           | kegg_pathway_89     |
| Focal adhesion                    | 5.47739          | 0.00418024         | 9                           | kegg_pathway_188    |
| Dilated cardiomyopathy            | 5.46448          | 0.00423454         | 6                           | kegg_pathway_263    |
| Regulation of actin cytoskeleton  | 5.43195          | 0.00437454         | 9                           | kegg_pathway_139    |
| PI3K-Akt signaling pathway        | 5.03748          | 0.00649008         | 12                          | kegg_pathway_262    |
| Shigellosis                       | 4.84404          | 0.00767516         | 5                           | kegg_pathway_82     |
| HTLV-I infection                  | 4.74205          | 0.00872078         | 10                          | kegg_pathway_190    |
ChIP-seq (Chromatin immunoprecipitation [ChIP] with massive parallel DNA sequencing data) [38], we characterized further how these transcriptional factors interact with candidate microRNAs in the MPM system. Here, we found that Jun/AP1 binds to the promoter region (defined by ChIPBase as 5 kb upstream and 1 kb downstream) of the following microRNAs: hsa-miR-30c-1, hsa-miR-30e, hsa-miR-20a, hsa-miR-27a-3p. Here, we found that Jun./AP1 binds to the promoter region (defined by ChIPBase as 5 kb upstream and 1 kb downstream) of the following microRNAs: hsa-miR-30c-1, hsa-miR-30e, hsa-miR-20a, hsa-miR-27a-3p.

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