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LETTER TO THE EDITOR

# Mechanosensitive pathways are regulated by mechanosensitive miRNA clusters in endothelial cells

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## Abstract

Shear stress is known to affect many processes in (patho-) physiology through a complex, multi-molecular mechanism, termed mechanotransduction. The sheer complexity of the process has raised questions how mechanotransduction is regulated. Here, we comprehensively evaluate the literature about the role of small non-coding miRNA in the regulation of mechanotransduction. Regulation of mRNA by miRNA is rather complex, depending not only on the concentration of mRNA to miRNA, but also on the amount of mRNA competing for a single mRNA. The only mechanism to counteract the latter factor is through overarching structures of miRNA. Indeed, two overarching structures are present miRNA families and miRNA clusters, and both will be discussed in details, regarding the latest literature and a previous conducted study focussed on mechanotransduction. Both the literature and our own data support a new hypothesis that miRNA-clusters predominantly regulate mechanotransduction, affecting 65% of signalling pathways. In conclusion, a new and important mode of regulation of mechanotransduction is proposed, based on miRNA clusters. This finding implicates new avenues for treatment of mechanotransduction and atherosclerosis.

**Keywords** Shear stress · Laser capture · miRNA families · Signalling pathways · Mechanotransduction

## Introduction

It is well known that the shape and size of blood vessels are determined by mechanical factors, like shear stress (Lu and Kassab 2011; Agrotou et al. 2013; Ghaffari et al. 2015). Shear stress is the friction force imposed onto the stationary endothelial cells by the movement of blood through the vessel. The biomechanical environment of endothelial cells is sensed through a complex process called mechanotransduction (Krizaj et al. 2014; Kshitiz et al. 2014; Liu and Lee 2014). This process consists of ~ 5000-7000 genes that are organised into > 40 signalling cascades which are regulated by > 8 mechanosensors (Han et al. 2004) and > 50 transcription factors (Qiao et al. 2016; Rajendran et al. 2016; Kunnen et al. 2018). The sheer complexity of mechanotransduction makes one wonder how it is regulated. Here, we aim to focus on post-translational control of signalling pathways by small non-coding RNA.

## Biogenesis and function of miRNA

miRNAs are short noncoding RNAs that regulate gene expression at the post-transcriptional level (Churov et al. 2019; Schafer and Ciaudo 2020). miRNAs are transcribed by RNA polymerase II (RNA pol II) in the nucleus to form pri-miRNAs, which are reduced in size into hairpin-shaped pre-miRNA by the Drosha–DGCR8 complex (Churov et al. 2019; Schafer and Ciaudo 2020). Pre-miRNA is exported from the nucleus into the cytoplasm by Ran-GTP and the Exportin-5 complex (Churov et al. 2019; Lopez-Pedrera et al. 2020; Schafer and Ciaudo 2020). In the cytoplasm, the pre-miRNA is cleaved by Dicer together with Argonaut (AGO) and trans-activation responsive RNA-binding protein (TRBP) to produce a double-stranded 20–25 nt miRNA (Churov et al. 2019; Lopez-Pedrera et al. 2020; Schafer and Ciaudo 2020).

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Subsequently, the miRNA duplex is incorporated into a multicomponent protein complex known as an RNA-induced silencing complex (RISC). In RISC, the 5′ strand of the miRNA duplex is selected while the other strand (miRNA-3p) is rapidly degraded (Churov et al. 2019; Lopez-Pedrera et al. 2020; Schafer and Ciaudo 2020). The single-stranded miRNA-5p acts as a scaffold for the complementary mRNA for destruction and/or for its translational repression via precise mechanisms (Liangju et al. 2015; Tao et al. 2015; Ballantyne et al. 2016; Feinberg and Moore 2016; Pastorkova et al. 2016).

The degree of repression of mRNA by miRNA is a rather complex process (Martirosyan et al. 2019). For a single miRNA-mRNA interaction, it depends on the level of expression of a miRNA and the abundance of the target mRNA (Figure 1A). As the mRNA-miRNA complex is destroyed, it is the relative concentration of both molecules which determines the degree of repression (Figure 1A). As one miRNA regulates 0–500 mRNA, reality is that mRNA targets compete for a single miRNA. Recently, elegant studies were performed examining how the number of target mRNAs per miRNA was affecting the degree of repression (Li et al. 2018; Abdollahzadeh et al. 2019; Martirosyan et al. 2019). These studies show that effectively, the more mRNAs regulated by a single miRNA are, the more competition there is leading to a dilution and a reduction in repression (Figure 1B). The presence of this effect has been discussed extensively in the literature and is the topic of a series of reviews (Li et al. 2018; Abdollahzadeh et al. 2019; Martirosyan et al. 2019). A method to counteract the competitive effect of the single miRNA-multiple mRNA hypothesis is by cooperativity of individual miRNA. In the last few years, a clear role for overarching structures in miRNA control, like miRNA families and miRNA clusters, have emerged as regulators of signalling pathways, or phenotypic changes that encompass groups of pathways (Przygrodzka et al. 2020; Rui et al. 2020; Singh et al. 2020) (Figure 1C).

## Evidence for an emerging role of families in mRNA repression

Families of miRNAs have been defined as miRNA sharing a common ancestor, or a common structural similarity situated in the seed region (Cantini et al. 2019; Moi et al. 2019; Srivastava et al. 2019). About 73% (15,554) of the miRNA genes in miRBase v19 have been assigned to 1543 miRNA families, further providing evidence for an important role of these families (Farahani et al. 2020). Interestingly, it has been observed that miRNA genes in the same miRNA family are non-randomly co-localized and well organized around genes involved in infectious, immune system, sensory system and neurodegenerative

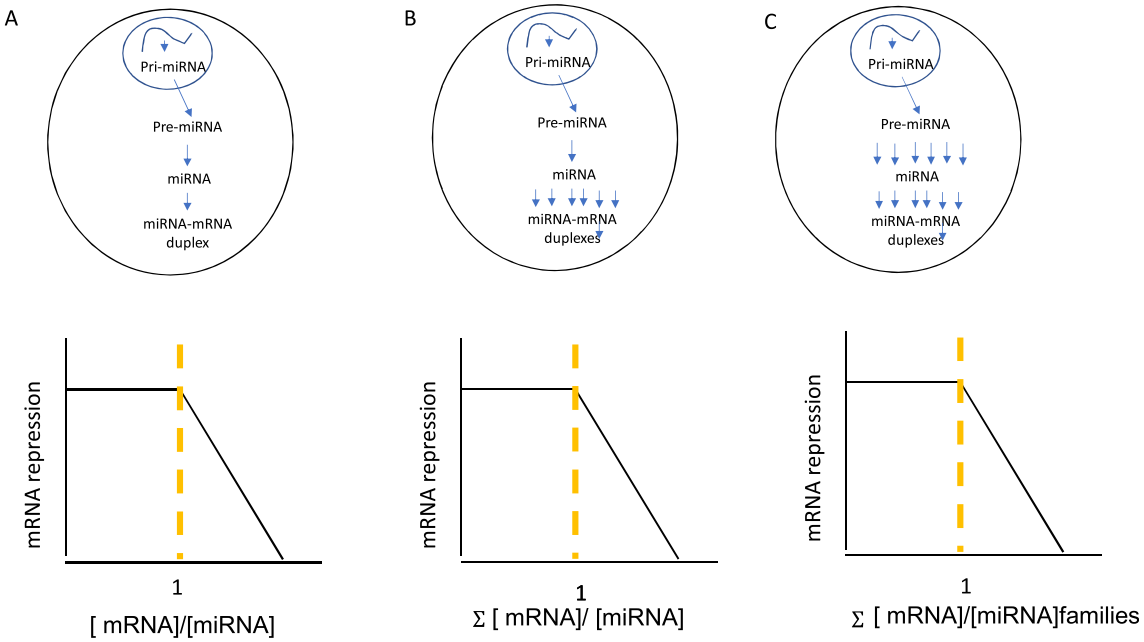


**Fig. 1** **A** describes the scheme how a single mRNA-miRNA duplex is leading to repression. Only when miRNA > mRNA or are at equal concentration is full repression possible. With mRNA > miRNA, partial repression is only possible. In **B**, the effect of multiple mRNA per miRNA is indicated competing for the same miRNA; **C** the coordination of families is indicated compensating for the competition of multiple mRNA and mRNA

**Table 1** Family is the miRbase-v22 defined family of miRNA, CLUSTER numbering as obtained from miRbase-v22, miRNA is the differentially expressed miRNA of that cluster, the pathway obtained, and size stands for the number of mRNA regulated by the cluster(s)

| Pathway | Families | Clusters |
|---|---|---|
| TGF-β signalling, Hedgehog, RB pathway, mTORC1 signalling | miR-17/92 | 7 |
| BH3-only protein Bim | miR-106b/25 | 25 |
| p21/cyclinD1 | miR-212/132 | 40 |
| KIT/ETV1 | miR-221/222 | 65 |
| PTEN/Akt | miR-144/451 | 52 |
| SMAD2 | miR-212/132 | 40 |
| Wnt/β-catenin | miR-17/92 | 7 |
| TGF-β signalling | miR-17/92; miR106b/25 | 7, 25 |
| Rho/ROCK | miR-200b/429 | 24 |
| p21/Bim | miR-106b/25 | 25 |
| KIT/ETV1 | miR-221/222; miR-17/92; | 7, 65 |

*AKT* AKT serine/threonine kinase, *BH3* Bcl-2 homology 3 domain, *BIM* Bcl-2-like protein 11, *EP300* E1A-associated protein p300, *ETV1* Ets variant gene 1, *KIT* proto-oncogene tyrosine-protein kinase, *MET* MET proto-oncogene receptor tyrosine kinase, *mTORC1* mammalian target of rapamycin complex 1, *P21* cyclin dependent kinase inhibitor 1A, *PLCG1* phospholipase c gamma 1, *PSAP* prosaposin, *P53* tumour protein P53, *PTEN*, phosphatase and tensin homolog, *RB1* RB transcriptional corepressor 1, *RHO* ROCK Rho-associated protein kinase, *SLU7* pre-mRNA splicing factor SLU7, *SMAD2* mothers against dpp homolog 2, *β-TRCP2* (also known as FBXW11), F-box and WD repeat domain containing 11, *TGF-β* transforming growth factor beta, *WEE1* Wee1A kinase *Wnt* wingless-type *mmtv* integration site family

diseases, development and cancer (Li and Mao 2007; Howe et al. 2012; Servin-Gonzalez et al. 2015; Granados-Lopez et al. 2017; Jiang et al. 2017; Balzano et al. 2018; Yin et al. 2018; Pinchi et al. 2019; Ferneza et al. 2021; Gregorova et al. 2021). The family members can vary from 2 to 30 members (e.g. Let-7). The larger miRNA families seem to be more conserved and are regulators of cell survival pathways, while "newer" families regulate more sophisticated processes, as the immunological response (Li and Mao 2007; Howe et al. 2012;

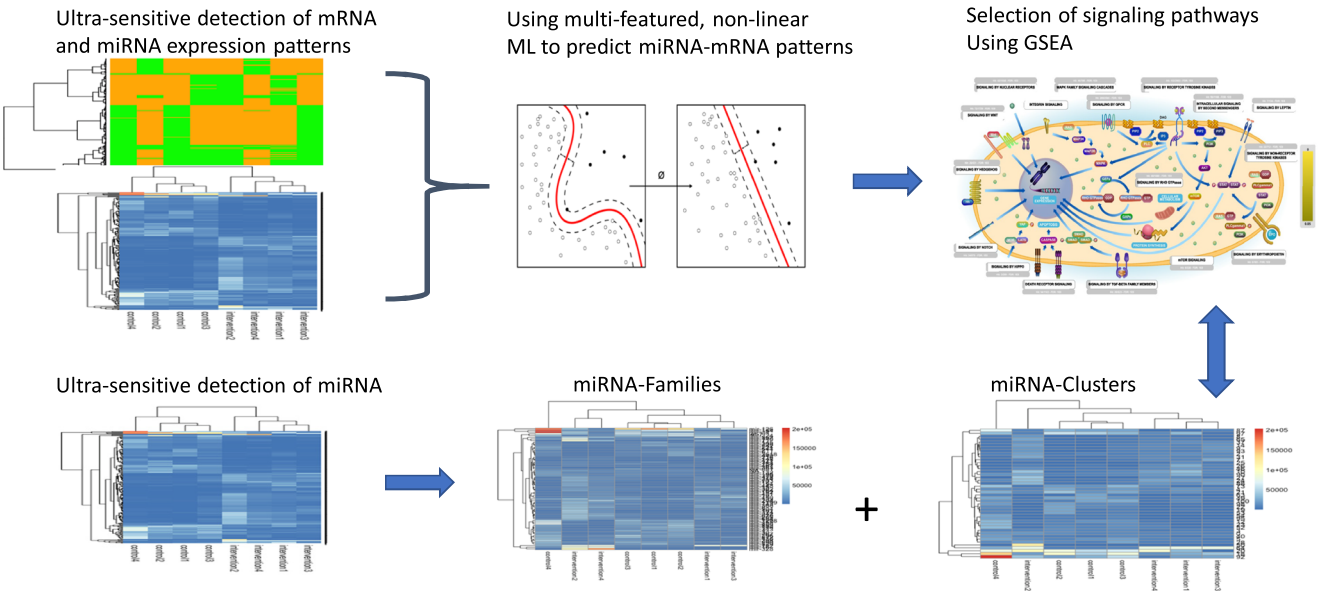


**Fig. 2** Schematic presentation of the proposed analysis of our data obtained from the laser captured endothelial cells. In the upper row on the left side is displayed the map of the top 100 genes and 215 microRNA. In the top row, middle panel is displayed the Support Vector Classifier scheme used to predict miRNA/mRNA interactions and the upper row, right panel shows one of the signalling pathways derived from our analysis. The lower panel shows the distribution of the miRNA, and the miRNA-families and miRNA-clusters derived from the differentially expressed miRNA. Not all maps are derived from real data

Servin-Gonzalez et al. 2015; Granados-Lopez et al. 2017; Jiang et al. 2017; Balzano et al. 2018; Yin et al. 2018; Pinchi et al. 2019; Ferneza et al. 2021; Gregorova et al. 2021).

The above-presented model (Figure 1) explaining why individual miRNAs exert only a modest effect on miRNA is based on a balance of expression of miRNA over mRNA (Figure 1A) versus a dilution effect of the competition of individual mRNA for a single miRNA (Figure 1B). On average, a single miRNA regulates ~40 mRNA, but this might vary from 0 to ~500 mRNA (Delahunty et al. 2020). Quantitatively, this means — as mRNA-miRNA dimers do not recycle — that the concentration of a single miRNA needs to be far higher (40–500 times) than their target mRNA to fully repress the abundance of the target mRNA. Since a large fraction of miRNAs are often co-regulated with their target genes, this high level of miRNAs is not always achievable. Here, we propose an alternative mechanism to control abundant mRNA targets, which is based on the recruitment of miRNA family members (Figure 1C). Furthermore, as individual members of a family are often situated in different chromosomes, they are differently regulated. As a consequence, not all members of a single family are activated at the same time to the same extent, and we propose that a gradual activation of miRNA families may lead to a gradual repression of their target mRNAs.

## Evidence for an emerging role of miRNA clusters in signalling pathways

MicroRNA clusters are individual microRNA positioned in close vicinity of each other, not separated by a transcription unit. Clusters consist both of members of families (homologous members) and structurally unrelated miRNAs (heterologous members) (Servin-Gonzalez et al. 2015; Gregorova et al. 2021). MirBase v22 identifies 100 clusters in the mouse genome and 156 in human genome, and their numbers are continuously increasing. The size of these clusters often varies between a few members to over > 10 members. Interestingly, they are often regulated as a functional unit through a polycistronic mechanism, consisting of either direct transcriptional control or directly through coding proteins (Servin-Gonzalez et al. 2015; Gregorova et al. 2021). At present, it is not clear how individual miRNA members and entire cluster interact. Several studies indicate that miRNAs within a single

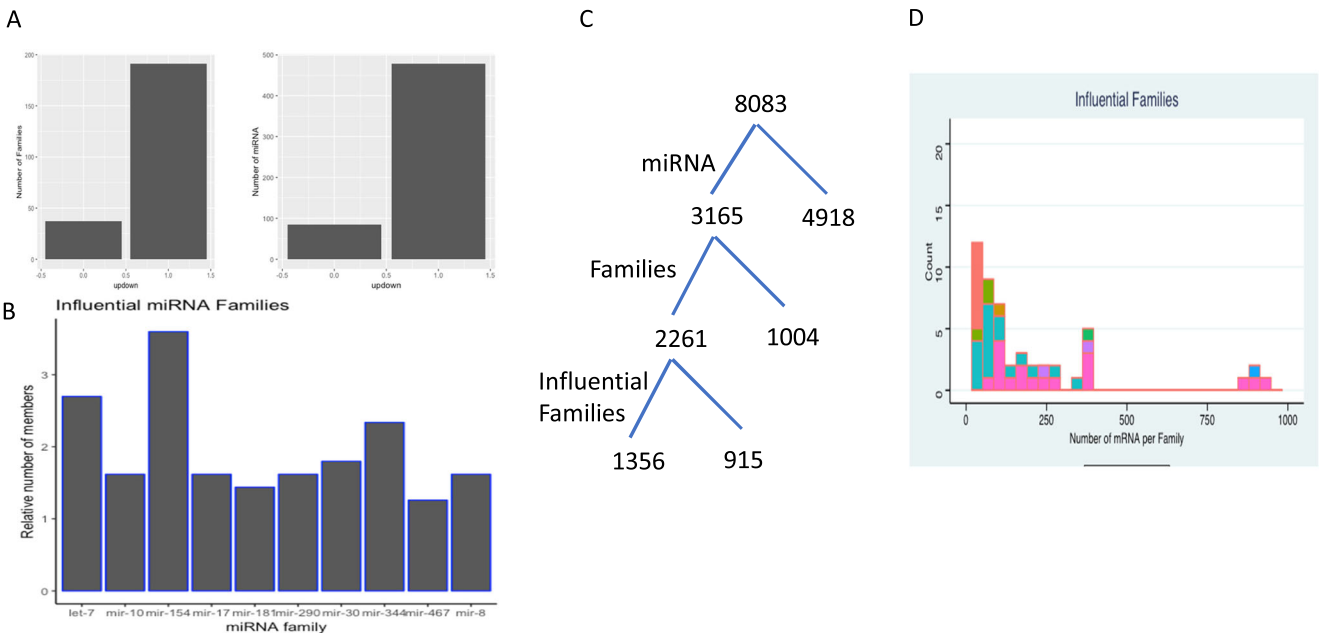


**Fig. 3** A detailed analysis of mechanosensitive miRNA families. **A** The number of differentially expressed mechanosensitive mRNA controlled by miRNA (3165), controlled by families (2261) and controlled by influential families (1365). **B** displays the distribution of mRNA regulated either by miRNA. In colour is displayed the lowest one third (red), middle one third (green) and upper third (blue) of mRNA per miRNA for the miRNA. The same colour coding has been introduced for miRNA families. Note that influential miRNA families contain more influential miRNA. **C** shows the distribution of families per mRNA regulated per family. The colour displays the number of influential miRNA per family.

cluster regulate each other, probably increasing the homogeneity in their response to stimuli. Other studies have emerged, indicating that miRNA clusters regulate one or more signalling pathways (Table 1). It is clear from Table 1 that either one cluster may regulate one pathway, or multiple clusters may regulate a single pathway, or one cluster may regulate multiple pathways (Table 1). In the latter observation, miRNAs are involved in processes, like immunological reactions, lipid handling and metabolic responses (Servin-Gonzalez et al. 2015; Gregorova et al. 2021).

## miRNA families and clusters regulate the majority of mechanosensitive mRNA in vivo

It is well known that the shape, size and physiology of blood vessels are determined by mechanical factors, like shear stress (Lu and Kassab 2011; Agrotou et al. 2013; Ghaffari et al. 2015). It also plays an important role in diseases like atherosclerosis. The biomechanical environment of endothelial cells is sensed through a complex process called mechanotransduction (Krizaj et al. 2014; Kshitiz et al. 2014; Liu and Lee 2014). This process consists of ~ 5000–7000 genes that are organised into > 40 signalling cascades which are regulated by > 8 mechanosensors (Han et al. 2004) and > 50 transcription factors (Qiao et al. 2016; Rajendran et al. 2016; Kunnen et al. 2018). The sheer complexity of mechanotransduction makes one wonder how it is regulated. Here, we aim to focus on post-translational control of signalling pathways by small non-coding RNA.

Several reviews have been written on mechanosensitive epigenetics and microRNA (Loyer et al. 2015; Nishiguchi et al. 2015; Feinberg and Moore 2016; Giral et al. 2016; Lu et al. 2018; Fasolo et al. 2019; Lee and Chiu 2019; Lopez-Pedrera et al. 2020). In these studies, quite often cultured endothelial cells were used with the exception of studies from Dr. Jo's group. Most reviews summarised the state of the field, providing evidence for an increasing role of microRNA in regulating microRNA. Indeed, over the years, the microRNA regulating mechanotransduction increased from ~ 15 to ~ 75 (Marin et al. 2013; Neth et al. 2013; Kumar et al. 2014; Wang et al. 2015; Fernandez Esmerats et al. 2016; Lee and Chiu 2019). And as a single miRNA affects ~ 40 mRNA, their influence increased, affecting ~ 3000

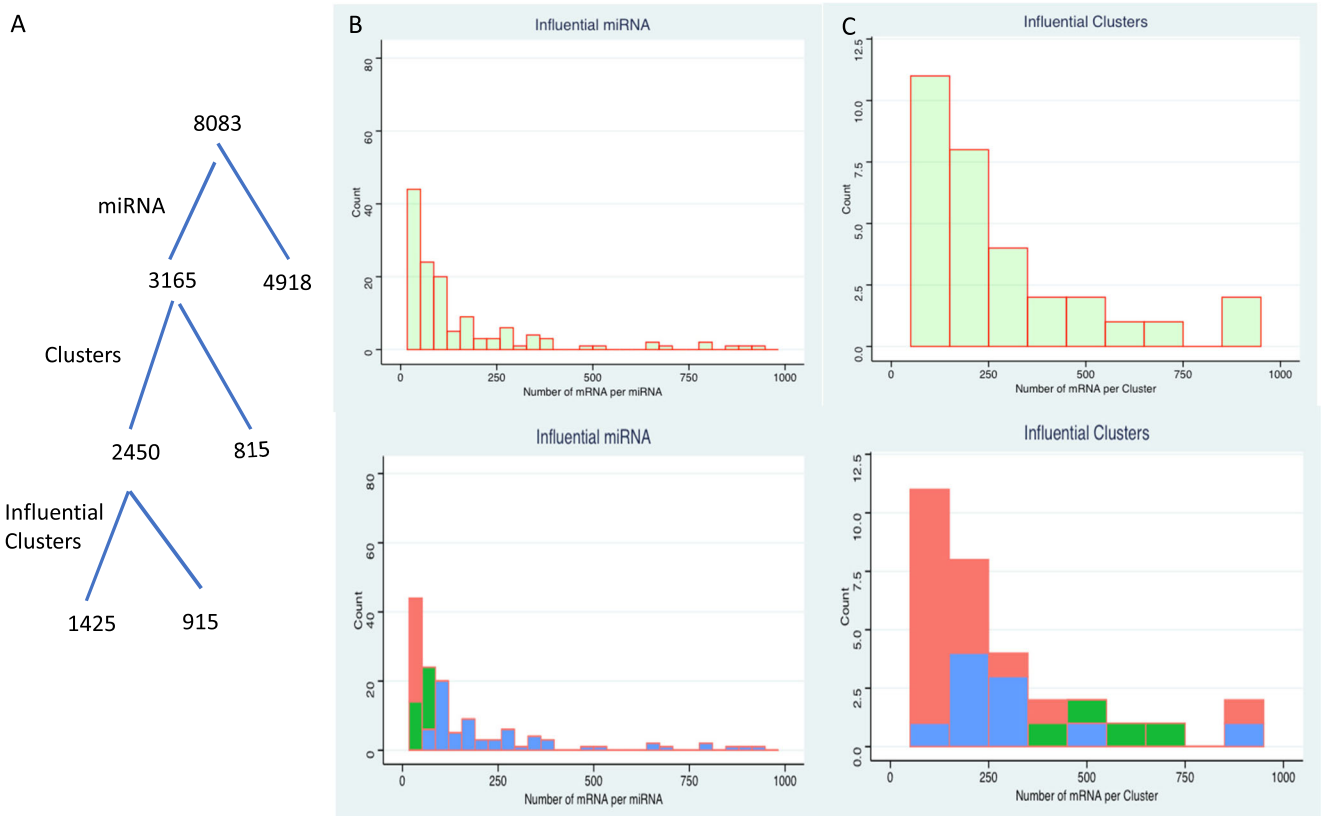


**Fig. 4** A detailed analysis of mechanosensitive miRNA clusters. **A** displays the distribution of mRNA regulated by miRNA (3165) and by clusters (2450) and by influential clusters (1425). In **B**, the distribution of individual miRNA per mRNA is displayed (upper row). The graph is coloured according to miRNA with lower third of mRNA regulation (red colour), middle third of mRNA (blue colour) and highest number of mRNA regulation (blue colour). In the lower row, a similar colour distribution is applied to the distribution of clusters. It is clear that the influential clusters contain more influential miRNA (more blue colour)

**Table 2** Family is the miRbase-v22 defined family of miRNA, CLUSTER numbering as obtained from miRbase-v22, miRNA is the differentially expressed miRNA of that cluster, the pathway obtained, and size stands for the number of mRNA regulated by the cluster(s)

| Family | Cluster | miRNAs | Pathways |
|---|---|---|---|
| mir-154 | 2 | mmu-miR-329-3p | Prostacyclin |
| mir-329 | | mmu-miR-376a-3p | PPAR |
| mir-368 | | mmu-miR-376c-3p | |
| mir-379 | | mmu-miR-380-5p | |
| mir-154 | 2 | mmu-miR-329-3p | Ubiquitin Proteasome |
| mir-329 | | mmu-miR-376a-3p | |
| mir-368 | | mmu-miR-376c-3p | |
| mir-379 | | mmu-miR-380-5p | |
| mir-471 | 3 | mmu-miR-471-3p | G-protein signalling |
| mir-742 | | mmu-miR-742-5p | |
| mir-743 | | mmu-miR-743a-3p | |
| mir-881 | | mmu-miR-881-5p | |
| mir-883 | | mmu-miR-883a-3p | |
| mir-431 | 4 | mmu-miR-3071-3p | Metabolism |
| | | mmu-miR-3071-5p | |
| | | mmu-miR-431-5p | |
| mir-290 | 5 | mmu-miR-291a-5p | Nima kinases |
| | | mmu-miR-292b-5p | Protein breakdown |
| | | mmu-miR-293-3p | |
| | | mmu-miR-294-3p | |
| mir-19 | 7 | mmu-miR-19b-3p | Metabolism |
| | | | WnT |
| mir-302 | 9 | mmu-miR-302b-3p | Prostacyclin |
| | | | eNOS |
| | | | Drug metabolism |
| mir-344 | 12 | mmu-miR-344e-5p/mmu-miR-344h-5p | Mechanosensors |
| | | mmu-miR-344f-3p | |
| | | mmu-miR-344i | |
| let-7 | 18 | mmu-let-7d-3p | G-protein signalling |
| | | mmu-let-7f-2-3p | |
| let-7 | 20 | mmu-let-7e-5p | RAF-MAPK |
| mir-10 | | mmu-miR-125a-5p | |
| mir-133 | 21 | mmu-miR-133a-5p | RAF-MAPK |
| mir-1 | | mmu-miR-1a-3p | |
| mir-133 | 21 | mmu-miR-133a-5p | JAK-STAT |
| mir-1 | | mmu-miR-1a-3p | Cytokine to cytokine |
| mir-8 | 24 | mmu-miR-200a-3p | Scavenger receptors |
| | | mmu-miR-200c-3p | PPAR pathway |
| mir-17 | 25 | mmu-miR-106b-3p | GAG and carbon metabolism |
| mir-182 | 26 | mmu-miR-182-3p | Metabolism |
| mir-133 | 28 | mmu-miR-133b-3p | G-protein |
| mir-1 | | mmu-miR-206-3p | Cytokine-to-cytokine |
| | | mmu-miR-206-5p | |
| mir-214 | 31 | mmu-miR-214-5p | G-protein signalling |
| | | | WnT |
| mir-216 | 37 | mmu-miR-216b-3p | Prostacycline |
| | | mmu-miR-216c-3p | eNOS |
| | | | Calcium-metabolism |
| | | | Pyrimidine-metabolism |
| mir-132 | 40 | mmu-miR-132-3p | ILP3-inflammasome |
| | | mmu-miR-132-5p | ROS |
| mir-15 | 46 | mmu-miR-15a-5p | Metabolism |
| | | mmu-miR-15b-3p | |
| let-7 | 50 | mmu-let-7c-5p | G0-G1 division |
| mir-122 | 64 | mmu-miR-122-3p | Chemokine binding receptors |
| mir-296 | 73 | mmu-miR-296-5p | Cell division |
| mir-298 | | mmu-miR-298-3p | Cytokine pathway |
| miR-1199 | 88 | mmu-miR-1199-3p | Prostacyclin |

**Table 2** (continued)

| Family | Cluster | miRNAs | Pathways |
|---|---|---|---|
| | | | eNOS<br>PAF |
| mir-34 | 92 | mmu-miR-34a-5p | Osteoclast differentiation<br>WnT |
| mir-767 | 98 | mmu-miR-767 | G-protein, calmodulin |
| LET-7 | 100 | mmu-miR-98-3p<br>mmu-miR-98-5p | Neurotrophin signalling<br>Protein breakdown<br>WnT |

*AKT* AKT serine/threonine kinase, *BH3* Bcl-2 homology 3 domain, *BIM* Bcl-2-like protein 11, *EP300* E1A-associated protein p300, *ETV1* Ets variant gene 1, *KIT* proto-oncogene tyrosine-protein kinase, *MET* MET proto-oncogene receptor tyrosine kinase, *mTORC1* mammalian target of rapamycin complex 1, *P21* cyclin dependent kinase inhibitor 1A, *PLCG1* phospholipase c gamma 1, *PSAP* prosaposin, *P53* tumour protein P53, *PTEN* phosphatase and tensin homolog, *RB1* RB transcriptional corepressor 1, *RHO* ROCK Rho-associated protein kinase, *SLU7* pre-mRNA splicing factor SLU7, *SMAD2* mothers against dpp homolog 2, *β-TRCP2* (also known as FBXW11) F-box and WD repeat domain containing 11, *TGF-β* transforming growth factor beta, *WEE1* Wee1A kinase, *Wnt* wingless-type mmtv integration site family

mRNA or ~ 50–60% of mechanotransduction, similar to their reported effect on the entire genome (Marin et al. 2013; Neth et al. 2013; Kumar et al. 2014; Wang et al. 2015; Fernandez Esmerats et al. 2016; Lee and Chiu 2019).

Families and clusters have sporadically (e.g. miR17-92 and Let-7) been mentioned as regulators in these reviews of mechanotransduction, but a systematic study was lacking. Considering the above arguments which stimulated us to study their roles in more detail (Figure 2) (Alex-Jade Delahunty1*, 2020), we identified that 224 families (miRbase-v22) were mechanosensitive, of which 187 miRNA-families were downregulated and 37 families were upregulated by a 7-day reduction of shear stress (Figure 3A).

Interestingly, a large fraction (~ 65%) of miRNA-depended mRNA was regulated by miRNA-family (Figure 3C). Bootstrapping identified 10 miRNA-families comprising only ~ 20% of all miRNA (Figure 3B), which regulated ~ 40% of the 65% of miRNA-regulated mechanosensitive mRNA (Figure 3C). These influential miRNA families exerted their influence, not by having more miRNA, but by having more "influential" miRNA members (Figure 3D: $p < 0.05$ bootstrapping). Influential miRNAs were defined as the miRNA regulating the highest third of number of mRNAs. The average mRNA regulated by all miRNA was 40, while that of the influential miRNA was 100 ($p < 0.05$).

We selected clusters on the basis of a strict criterion of < 3000 kB proximity (Wang et al. 2019) and identified that 35% of mechanosensitive miRNAs are organised in 43 clusters (miRBase v22) which is slightly higher than reported for the entire murine genome (28% of the miRNA (Wang et al. 2019)). We subsequently confirmed that mechanosensitive clusters are regulated by a polycistronic mechanism (Wang et al. 2019), e.g. their variance in expression was lower in a cluster than between cluster ($p < 0.05$), indicating they are functionally controlled as well (Alex-Jade Delahunty1*, 2020).

Interestingly, a very large fraction (~ 60%) of mechanosensitive mRNA controlled by miRNA was regulated by the clustered miRNA (35% of all miRNA: Figure 4A). Similarly, as for families, clusters are enriched with influential miRNA ($p < 0.05$) and influential miRNA-families ($p < 0.05$: Figure 4B) providing a basis for this large number of mRNA (Alex-Jade Delahunty1*, 2020).

## A central role for clusters in mechanosensitive pathway coordination

The 7-day reduction in shear stress induced 8083 mechanosensitive genes and 215 miRNA (FDR < 0.05), the largest number of differentially expressed mRNA and miRNA to date. Gene set Enrichment analysis (GSEA) (Ding et al. 2018; Xiong et al. 2018; Zhu et al. 2018; Alsagaby 2019; Chung et al. 2019; Kowsar et al. 2019) identified > 100 mechanosensitive pathways, of which the most prominent were (i) metabolism of genes and proteins, (ii) extracellular matrix genes, (iii) programmed cell death and (iv) signal transduction (Alex-Jade Delahunty1*, 2020). A further, focussed analysis of the signal transduction pathways revealed that 41 signalling pathways were affected by the reduction in shear stress. These included well-known shear stress–sensitive pathways such as eNOS and MAPK (Wang et al. 2014; Riquelme et al. 2015; Lee et al. 2017; Kunnen et al. 2018), recently established mechanosensitive pathways such as NOTCH and WnT (Kuo et al. 2015; Kuo et al. 2015; Jia et al. 2018; Kunnen et al. 2018; Xu et al. 2018; Yang et al. 2018; Bondareva et al. 2019; Gater et al. 2019; Han et al. 2019; Kouzbari et al. 2019; Varshney et al. 2019; Yue et al. 2019) and currently unknown mechanosensitive pathways like insulin (Table 2).

As discussed above (Table 1), it has been postulated that miRNA clusters regulate processes that involve one

or more signalling pathways, like immunological processes. For instance, differentiation of cardiomyocytes from precursor cells need waves of transcription factor activation which is regulated by miRNA clusters. Stimulated by these findings, we performed a further analysis of our miRNA-clusters which revealed that twenty-six (26) out of 43 differentially expressed miRNA clusters regulated 30 out of 41 (65%, $p < 0.05$) signalling pathways (Table 2) (Alex-Jade Delahunty1*, 2020). We found single clusters affecting a single pathway (clusters 2, 5), but the majority of clusters affected multiple pathways (Table 2). The latter finding has been identified before (see above) in embryology where affected pathways were related to processes, like development of organs (Cantini et al. 2019; Liu and Wang 2019; Hutter et al. 2020; Kandettu et al. 2020; Shukla et al. 2020; Zhang et al. 2020). In addition, multiple clusters could affect a single pathway (clusters 4,7, 26, 46 affecting metabolism), groups of clusters affecting physiological processes like vasodilation (clusters 9, 37, 88 affecting eNOS and prostacyclin, Table 2) while clusters regulating aspects of inflammation appeared more distributed (cluster 21, MAPK and JAK-STAT pathways, cluster 40, the inflammasome and cluster 73, the cytokine pathways) (Alex-Jade Delahunty1*, 2020).

In conclusion, while it has always been argued that mechanotransduction is regulated by master switches, transcription factors like KLF2 and KLF4 which regulate the majority of mechanosensitive genes, we present data here that microRNA clusters have a presently unknown, but profound effect on mechanotransduction. The precise coordination of these clusters needs further studies.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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