Supplementary figures and supplementary table legends

Pathway analysis from lists of microRNAs : common pitfalls and alternative strategy

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Supplementary information

The microRNA knowledge is biased for cancer biology

Many pathways identified by the strategy 1 are related to cancer which indicates a knowledge bias in this field for microRNA compared to protein coding genes.

To test this hypothesis, the relative representations of miRNA genes and protein coding genes in cancer-related scientific literature were compared using the SCAIview test mining tool(1). This tool allows a quantitative analysis of ontology-mapped terms co-occurence in the entire collection of Pubmed abstracts and extracted all Mesh diseases co-cited with either a miRNA or a protein coding genes.

Using an hypergeometric test, 1093 Mesh diseases were identified as significantly enriched with miRNA and 3320 with protein coding genes. Among them 17.5% (163) and 8.5% (259) respectively are neoplastic diseases. The difference between the proportions of neoplastic diseases associated to miRNA and protein coding genes is highly significant according to a Fisher test (p-value < 10^{-10}). This result shows a significant bias in current the knowledge related to the role of miRNA in diseases towards neoplastic and cancer-related processes. As recently discussed by Mørk(2), this bias could be related to a study imbalance or to the underlying function of miRNA as such. Anyway it has to be taken into account when performing analyses using the current knowledge, such as pathways analyses.

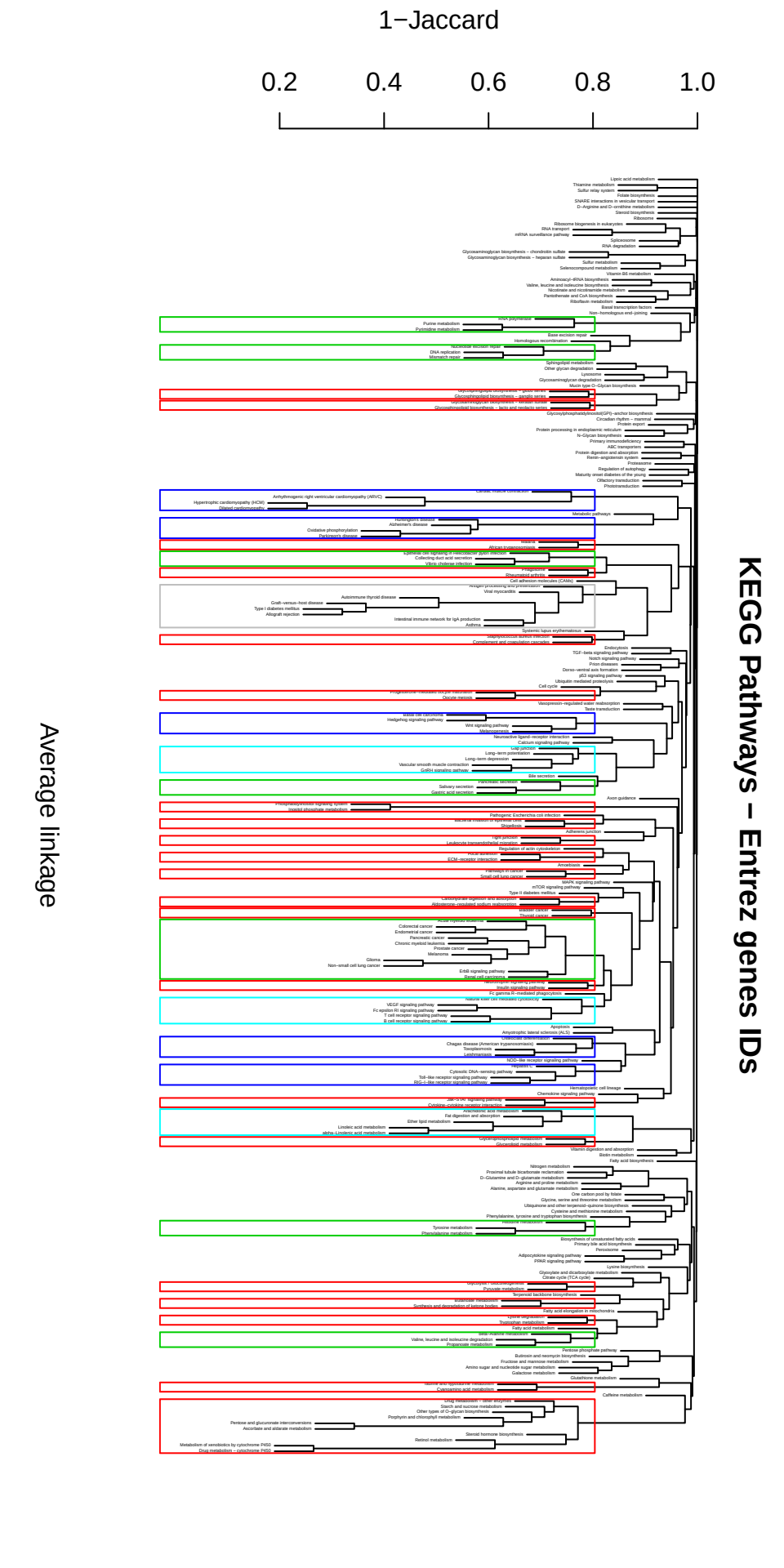
Method for literature analysis

The list of Mesh diseases co-cited with either protein coding genes or miRNA genes was obtained using SCAIview Pharma 1.0 running on the collection of Pubmed abstracts mentioning a Mesh disease as available on 19 November 2013 (13,145,693 abstracts). Mesh diseases significantly co-cited (FDR < 0.001) with either a protein coding gene or a miRNA were obtained applying an hypergeometric test.

To differentiate neoplastic Mesh diseases from other Mesh diseases, the Mesh disease ontology(3) was downloaded from Mesh website (http://www.nlm.nih.gov/mesh/2014/download/mtr_smp.txt) and all diseases falling under the node “C04 – Neoplasms” were classified as neoplastic compared to others.

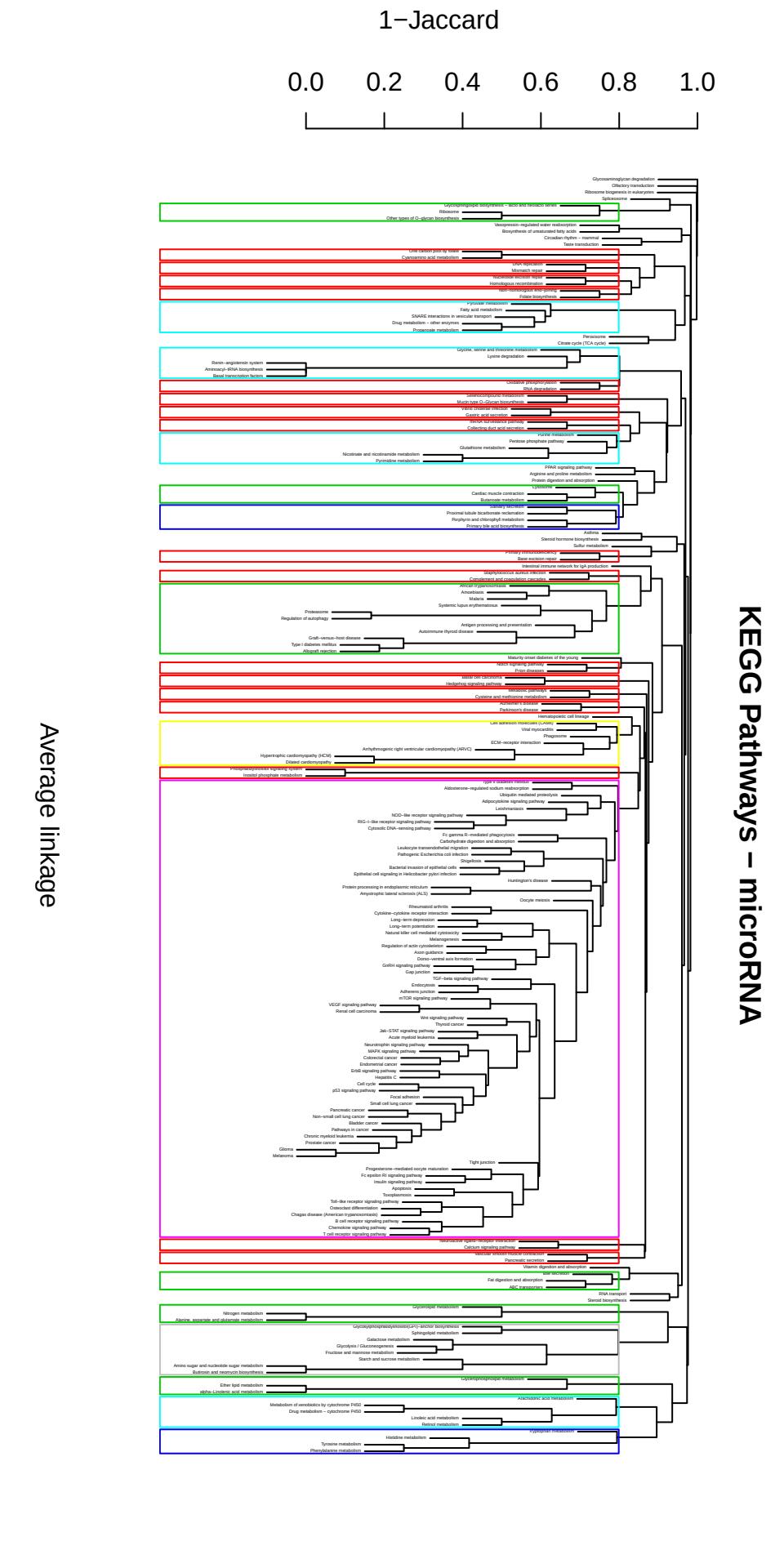
Supplementary Figure 1

Hierarchical clustering on the Jaccard index computed for each pathway couple at the level of protein coding gene. The average linkage method was used to build the tree. Cluster of pathways sharing on average at least 20% of Entrez gene ID according to the KEGG.db(4) package are highlighted.


1−Jaccard
0.2
0.4
0.6
0.8
1.0
Average linkage
KEGG Pathways – Entrez genes IDs

Supplementary Figure 2

Hierarchical clustering on the Jaccard index computed for each pathway couple at the level of microRNA. The average linkage method was used to build the tree. Cluster of pathways sharing on average at least 20% of associated miRNAs using mirTarBase(5) information are highlighted.


1−Jaccard
0.0
0.2
0.4
0.6
0.8
1.0
Average linkage
KEGG Pathways – microRNA
Glycosaminoglycan degradation
Olfactory transduction
Ribosome biogenesis in eukaryotes
Spliceosome
Glycosphingolipid biosynthesis – ganglio and lacto series
Ribosome
Other types of O−glycan biosynthesis
Vasopressin−regulated water reabsorption
Biosynthesis of unsaturated fatty acids
Circadian rhythm − mammal
Taste transduction
Cell cycle
Cysteine and methionine metabolism
DNA replication
Mismatch repair
Nucleotide excision repair
Homologous recombination
Non−homologous end−joining
Folate biosynthesis
Peroxisome
Fatty acid metabolism
SNARE interactions in vesicular transport
Drug metabolism − other enzymes
Propanoate metabolism
Citrate cycle (TCA cycle)
Glycine, serine and threonine metabolism
Lysine degradation
Renin−angiotensin system
Aminoacyl−tRNA biosynthesis
Basal transcription factors
Oxidative phosphorylation
RNA degradation
Selenocompound metabolism
Mucin type O−Glycan biosynthesis
Vibrio cholerae infection
Gastric acid secretion
Renin secretion
Collecting duct acid secretion
Purine metabolism
Pentose phosphate pathway
Glutathione metabolism
Nicotinate and nicotinamide metabolism
Pyrimidine metabolism
PPAR signaling pathway
Arginine and proline metabolism
Protein digestion and absorption
Cytokine
Cardiac muscle contraction
Butanoate metabolism
Salivary secretion
Proximal tubule bicarbonate reclamation
Porphyrin and chlorophyll metabolism
Primary bile acid biosynthesis
Asthma
Steroid hormone biosynthesis
Sulfur metabolism
Primary immunodeficiency
Base excision repair
Intestinal immune network for IgA production
Staphylococcus aureus infection
Complement and coagulation cascades
African trypanosomiasis
Amoebiasis
Malaria
Systemic lupus erythematosus
Proteasome
Regulation of autophagy
Antigen processing and presentation
Autoimmune thyroid disease
Graft−versus−host disease
Type I diabetes mellitus
Allograft rejection
Maturity onset diabetes of the young
Notch signaling pathway
Prion diseases
Basal cell carcinoma
Hedgehog signaling pathway
Metabolic pathways
Cysteine and methionine metabolism
Alzheimer's disease
Parkinson's disease
Hematopoietic cell lineage
Cell adhesion molecules (CAMs)
Viral myocarditis
Phagosome
ECM−receptor interaction
Arrhythmogenic right ventricular cardiomyopathy (ARVC)
Hypertrophic cardiomyopathy (HCM)
Dilated cardiomyopathy
Phosphatidylinositol signaling system
Inositol phosphate metabolism
Type II diabetes mellitus
Aldosterone−regulated sodium reabsorption
Ubiquitin mediated proteolysis
Adipocytokine signaling pathway
Leishmaniasis
NOD−like receptor signaling pathway
RIG−I−like receptor signaling pathway
Cytosolic DNA−sensing pathway
Fc gamma R−mediated phagocytosis
Carbohydrate digestion and absorption
Leukocyte transendothelial migration
Pathogenic Escherichia coli infection
Shigellosis
Bacterial invasion of epithelial cells
Epithelial cell signaling in Helicobacter pylori infection
Huntington's disease
Protein processing in endoplasmic reticulum
Amyotrophic lateral sclerosis (ALS)
Oocyte meiosis
Rheumatoid arthritis
Cytokine−cytokine receptor interaction
Long−term depression
Long−term potentiation
Natural killer cell mediated cytotoxicity
Melanogenesis
Regulation of actin cytoskeleton
Axon guidance
Dorso−ventral axis formation
GnRH signaling pathway
Gap junction
TGF−beta signaling pathway
Endocytosis
Adherens junction
mTOR signaling pathway
VEGF signaling pathway
Renal cell carcinoma
Wnt signaling pathway
Thyroid cancer
Jak−STAT signaling pathway
Acute myeloid leukemia
Neurotrophin signaling pathway
MAPK signaling pathway
Colorectal cancer
Endometrial cancer
ErbB signaling pathway
Hepatitis C
Cell cycle
p53 signaling pathway
Focal adhesion
Small cell lung cancer
Pancreatic cancer
Non−small cell lung cancer
Bladder cancer
Pathways in cancer
Chronic myeloid leukemia
Prostate cancer
Glioma
Melanoma
Tight junction
Progesterone−mediated oocyte maturation
Fc epsilon RI signaling pathway
Insulin signaling pathway
Apoptosis
Toxoplasmosis
Toll−like receptor signaling pathway
Osteoclast differentiation
Chagas disease (American trypanosomiasis)
B cell receptor signaling pathway
Chemokine signaling pathway
T cell receptor signaling pathway
Neuroactive ligand−receptor interaction
Calcium signaling pathway
Vascular smooth muscle contraction
Pancreatic secretion
Vitamin digestion and absorption
Bile secretion
Fat digestion and absorption
ABC transporters
RNA transport
Steroid biosynthesis
Glycerolipid metabolism
Nitrogen metabolism
Alanine, aspartate and glutamate metabolism
Glycosylphosphatidylinositol(GPI)−anchor biosynthesis
Sphingolipid metabolism
Galactose metabolism
Glycolysis / Gluconeogenesis
Fructose and mannose metabolism
Starch and sucrose metabolism
Amino sugar and nucleotide sugar metabolism
Butirosin and neomycin biosynthesis
Glycerophospholipid metabolism
Ether lipid metabolism
alpha−Linolenic acid metabolism
Arachidonic acid metabolism
Metabolism of xenobiotics by cytochrome P450
Drug metabolism − cytochrome P450
Linoleic acid metabolism
Retinol metabolism
Tryptophan metabolism
Histidine metabolism
Tyrosine metabolism
Phenylalanine metabolism

Supplementary table legends

Supplementary Table 1

Data associated to the miRNA signature of Alzheimer's disease (AD-up and AD-down) defined by Satoh 2012(6). Significance (FDR<0.05) is highlighted by red color in cells. Data are structured across different sheets as follows :

Original microRNA: miRNAs as provided in the original publication and their mapping in mirTarBase(5).

mirTarBase Targets: Entrez ID of genes targeted by AD-up and AD-down miRNAs according to mirTarBase.

AD-up S1 Pathways: Enrichment analysis results when applying strategy 1 on AD-up miRNAs. The first 3 columns are related to the significance of association with the original signature. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 1 to 1000 random selections of 16 miRNAs.

AD-down S1 Pathways: Enrichment analysis results when applying strategy 1 on AD-down miRNAs. The first 3 columns are related to the significance of association with the original signature. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 1 to 1000 random selections of 99 miRNAs.

AD-up S2 Pathways: Enrichment analysis results when applying strategy 2 on AD-up miRNAs. The first 3 columns are related to the significance of association with the original signature. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 2 to 1000 random selections of 16 miRNAs.

AD-down S2 Pathways: Enrichment analysis results when applying strategy 2 on AD-down miRNAs. The first 3 columns are related to the significance of association with the original signature. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 2 to 1000 random selections of 99 miRNAs.

AD-down S3 Pathways: Enrichment analysis results when applying strategy 3 on AD-down miRNAs. The first 3 columns are related to the significance of association with the original signature. The last 3 columns indicates to which cluster of Supplementary Figure 2 each pathway belongs, the number of significant pathways (FDR < 0.05)compared to the total number of pathways in the corresponding cluster. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 3 to 1000 random selections of 99 miRNAs.

Supplementary Table 2

All mirTarBase Targets S1 KEGG: KEGG pathways enriched in all protein coding genes targeted by at least one miRNA according to mirTarBase. The first 3 columns show the significance of the association. The remaining columns indicate pathways identified in different studies(6–9) focused on different topics.

Top-Targeted S2 KEGG: KEGG pathways enriched in genes targeted by at least one miRNA, in the top 1000, 500, 100 and 50 genes targeted by miRNAs according to mirTarBase. Only FDR is shown.

Supplementary Table 3

Data associated to the miRNA signature of Chordomas defined by Long *et al.* 2013(7). Significance (FDR<0.05) is highlighted by red color in cells. Data are structured across different sheets as follows :

Original microRNA: miRNAs as provided in the original publication and their mapping in mirTarBase(5).

Chordoma-up S3 Pathways: Enrichment analysis results when applying strategy 3 on Chordoma-up miRNAs. The first 3 columns are related to the significance of association with the original signature. The last 3 columns indicates to which cluster of Supplementary Figure 2 each pathway belongs, the number of significant pathways (FDR < 0.05)compared to the total number of pathways in the corresponding cluster. The remaining columns show the distribution of the FDR for each pathway when applying the strategy 3 to 1000 random selections of 19 miRNAs.

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