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RESEARCH ARTICLE

miRACA: A database for miRNAs associated with cancers and age related disorders (ARD)

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BACKGROUND: With the given diversity and abundance of several targets of miRNAs, they functionally appear to interact with several elements of the multiple cellular networks to maintain physiologic homeostasis. They can function as tumor suppressors or oncogenes, whose under or overexpression has both diagnostic and prognostic significance in various cancers while being implicated as prospective regulators of age-related disorders (ARD) as well. Establishing a concatenate between ARD and cancers by looking into the insights of the shared miRNAs may have a practical relevance.
METHODS: In the present work, we performed network analysis of miRNA-disease association and miRNA-target gene interaction to prioritize miRNAs that play significant roles in the manifestation of cancer as well as ARD. Also, we developed a repository that stores miRNAs common to both ARD and cancers along with their target genes.
RESULTS: We have comprehensively curated all miRNAs that we found to be shared in both the diseases in the human genome and established a database, miRACA (Database for microRNAs Associated with Cancers and ARD) that currently houses information of 1648 miRNAs that are significantly associated with 38 variants supported with pertinent data. It has been made available online at http://genomeinformatics.dtu.ac.in/miraca/ for easy retrieval and utilization of data by the scientific community.
CONCLUSION: To the best of our knowledge, our database is the first attempt at compilation of such data. We believe this work may serve as a significant resource and facilitate the analysis of miRNA regulatory mechanisms shared between cancers and ARD to apprehend disease etiology.

Keywords miRNA, cancer, age related disorders (ARD), target genes, database

Introduction

MicroRNAs (miRNAs) are naturally occurring small, approximately 22-nucleotides, non-coding endogenous RNA molecules that repress the expressions of genes (Bartel, 2004). They discriminately bind to the 3′-noncoding regions of the specific mRNAs through complementary base-pairing and hence suppress the translation of mRNAs or denature them (Esquela-Kerscher and Slack, 2006). A single miRNA target multiple mRNAs and so have the ability to alter the regulatory networks of gene expression significantly. The principle function of miRNA is to regulate various signal transduction networks permitting them to take control and facilitate robust transitions of cellular responses to extracellular signals. They are fundamental for various biological processes and as such have evoked significant scientific attention in the recent years. Intense studies and characterization of miRNAs have elucidated their interpretative functions in growth and development and the transformation of cellular responses to extracellular signals thereby maintaining physiologic homeostasis (Filipowicz et al., 2008; Huntzinger and Izaurralde, 2011). Various studies depicted defects in the miRNA biogenesis machinery to be a link to a range of disease mechanisms (Guo et al., 2010).

The potential of miRNAs to modulate aging process in model organisms has alluded the interest of the molecular genetics community to a greater extent. Like all other cellular processes, genes associated with aging are regulated by miRNAs which reveal the interconnected nature of transcript regulation involved in aging in humans and the role of miRNAs in this process (Gan et al., 2015; López-Otín et al., 2013). The association between miRNAs and longevity of life has been demonstrated in *C. elegans*, implicating their role in the regulation of longevity and eventually in the aging process (Lee et al., 1993). Deciphering the factors involved in

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degeneration of organism, tissue, and cellular homeostasis may provide biomarkers for healthy aging and expand the understanding of the processes that undergird aging.

Traditionally, the study of cancer has focused invariably on protein-coding genes contemplating these to be the principal effectors and modulators of tumorigenesis. However, recent advances in cancer biology have brought non-protein-coding RNAs or miRNAs into the limelight accentuating their importance in the regulation of cell growth, differentiation, and apoptosis (Hwang and Mendell, 2006). Many miRNA genes are in such genomic loci that are designated as fragile sites on chromosomes. These fragile sites are considered to be important as these are the preferred sites of translocation, deletion, amplification, or integration of exogenous genome and could be possible targets of genomic alterations (Lewis et al., 2005; Zhang et al., 2006). It is noteworthy that miRNAs remarkably show altered expression in cancers in correlation to normal tissues (Hanahan and Weinberg, 2000). This aids to recognize different physiologic locations of tumor formation and distinct specific signatures of prognostic expression which indicates that miRNAs can be exploited to serve as discreet prime mover of clinical belligerency (Iorio et al., 2005; Lu et al., 2005; Volinia et al., 2006). Therefore, there is unputdownable credible evidence that miRNAs are the master regulators of oncogenesis (Dalmay and Edwards, 2006). Nevertheless, these studies are still at a preliminary stage, and their functional mechanism of action along with their significance in cancer is not yet fully apprehended. One such perspective would be to seek supporting evidence of the contribution of miRNAs in the regulation of pathways and targets that are indeed strong logical candidates based on the current knowledge of cancer biology.

In the present work, we identified such specific miRNAs and their target genes and carried out network analysis of miRNA-disease association and miRNA-target gene interaction. Based on our network analysis results, we found significant hub miRNAs that might primarily influence the initiation and progression of both cancers and ARD. Such pertinent hub miRNAs can be explored and targeted for improved therapeutic interventions. Also, we found potential hub target genes that were found to be related with several miRNAs that are shared between cancers and ARD. We further analyzed the miRNA target genes for their respective functional, biological and molecular functions and pathways using bioinformatics methods to unravel its association in both the processes in an attempt to elucidate the prospective role of miRNAs in disease development. Furthermore, we have compiled all the relevant information that is scattered across published literature and online resources on miRNAs that we found to be significantly associated and shared in both the disease conditions and developed a database that hosts information on 1648 miRNAs along with their target genes in an attempt to make it convenient for researchers to thoroughly study the regulatory mechanisms of miRNAs in cancer and ARD. A graphical representation of the workflow is shown in Fig. 1.

Methodology

Data collection

The information of miRNA to analyze the role of miRNAs related to ARD and cancers was obtained from the online databases HMDD v2.0 (the Human microRNA Disease Database) (Li et al., 2014) and miRBase (Griffiths-Jones, 2006). We looked for ARD phenotypes in dbAARD (database of Aging and Age Related Disorders) (Srivastava et al., 2016) and its respective research article for the same and filtered the miRNAs associated with the ARD mentioned in the article.

On the other hand, we extracted the information of miRNAs associated will all the various forms of cancers from HMDD v2.0. A total of 436 types of miRNAs associated with ARD and 515 types of miRNAs associated with cancers were selected to construct the network.

Network construction for miRNA-disease association analysis

A structured network layout explaining network integrity is the core requirement to justify the interaction between miRNA and disease. Cytoscape 3.4.0 (Shannon et al., 2003) which is a free software package was used to visualize and analyze the disease-miRNA networks. The datasheet prepared which included all the miRNAs associated with ARD and cancers was used to generate the network. After obtaining the hub miRNA and disease, screening and analysis were performed to identify the target genes of the associated miRNAs.

Identifying shared miRNAs associated with both ARD and cancers

To establish a concatenate connection between ARD and cancers with respect to the role that miRNAs play, it is important to determine the shared miRNAs that are related to and have a fundamental role in the disease etiology of both ARD and cancers. The shared or common miRNAs were extracted using BioVenn (Hulsen et al., 2008) which is a free online software application for the comparison and visualization of biological lists using Venn diagrams defined being area-proportional. It summarizes the overlap between two or three lists of identifiers using area-proportional Venn diagrams. A total of 416 unique sharedmiRNAs were extracted using BioVenn that bridged a high solder connection between ARD and cancers.

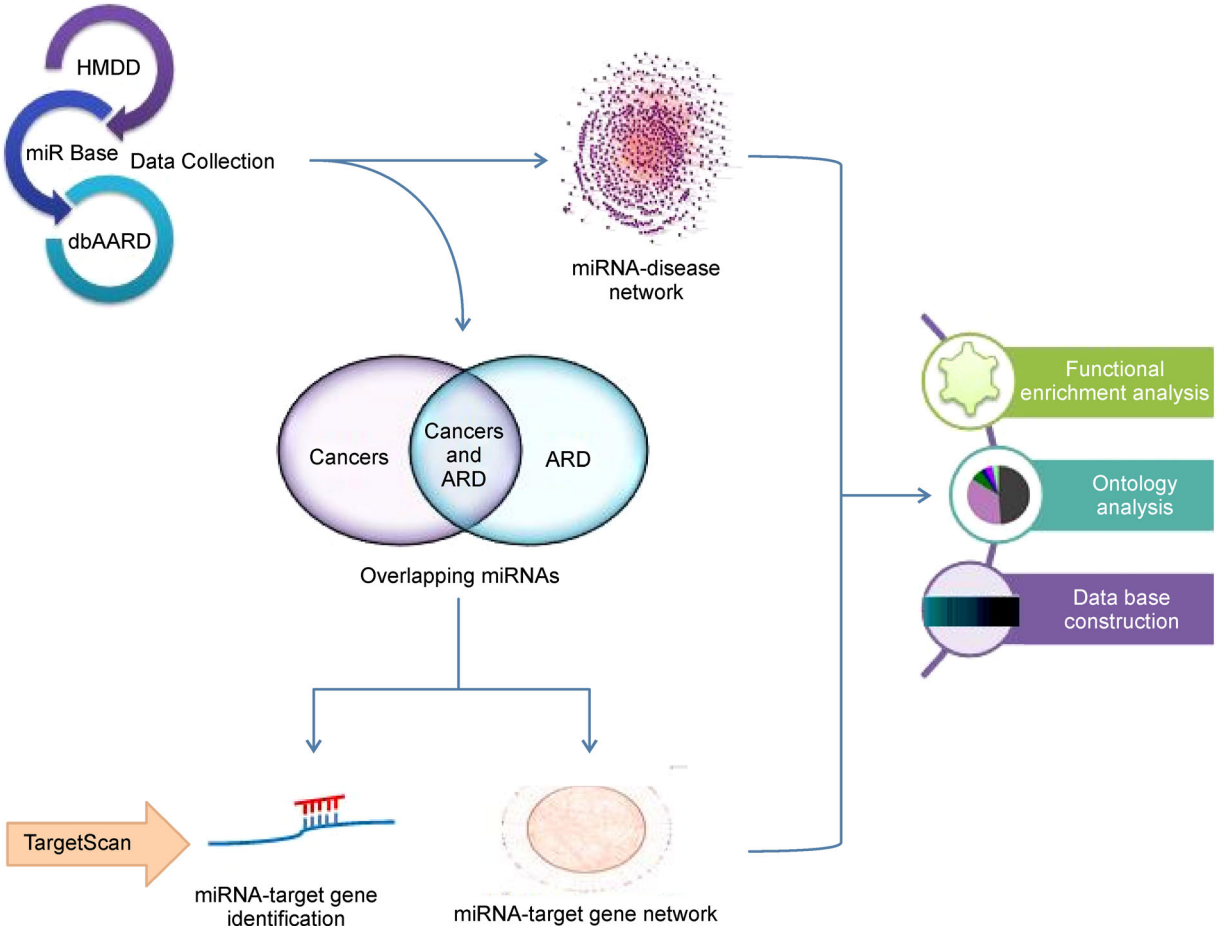


Figure 1 Graphical representation of the workflow.

Identifying target genes of the shared miRNAs common in both ARD and cancers

The shared miRNAs associated with both ARD and cancers that were identified were used to find their respective target genes. TargetScanHuman 7.1 (Agarwal et al., 2015), which is free online web tool, was used to detect targets in the 3′UTR of protein-coding transcripts by base-pairing rules where predictions with both broadly conserved and poorly conserved sites are also provided. These target sites are the conserved sites that match or are complementary to the seed region of the miRNA that ultimately facilitates the binding of miRNA with the mRNA to functionally degrade it (Wu et al., 2006).

Network construction for miRNA-target gene interaction analysis

A miRNA-target gene interaction network was constructed of the shared miRNAs and analyzed to understand the relationship between miRNA and target genes and validate the miRNA-disease interaction results in both the cases of ARD and cancers.

Functional enrichment analysis of miRNA target genes

To understand the miRNA-target genes relationship, we measure the individual relationships of the target genes based on the degrees of their co-association with diseases. Statistical approaches to identify the clusters or classes of proteins that are highly expressed or enriched in a large set of proteins can reveal meaningful biological interactions. Functional enrichment analysis of the miRNA target genes was carried out by DAVID (The Database for Annotation, Visualization and Integrated Discovery) v6 (Huang et al., 2009a, 2009b), which is an online database of web-accessible programs used to identify the groups of genes that are highly expressed or enriched in the entire gene list and to interpret their association with disease phenotypes.

Ontology analysis

Considering that a significant fraction of the genes specific for core biological functions is shared by all eukaryotes, the information about the shared genes and proteins facilitates our apprehension of all the diverse organisms that share them. Central Gene Ontology Consortium server, is a widely

accepted source of gene functional annotation, collaborating many databases that facilitate uniform queries across all of them. Thus, it was used to describe and analyze the molecular functions, biological processes and pathways for the selected target gene set of interest in which they were significantly involved. Also, PANTHER (Protein Analysis Through Evolutionary Relationships) classification system (Mi et al., 2005, 2013) which offers spontaneous visualization of images of GO analysis was used.

Construction of miRACA

The primary data in miRACA represents the association of shared miRNAs in both cancers and ARD. The information for the purpose of this work was obtained from existing database HMDD (Li et al., 2014), miRBase (Griffiths-Jones, 2006), and dbAARD (Srivastava et al., 2016). The miRNAs extracted from the existing databases were manually curated and compiled from the relevant articles published in PubMed and high-quality journals discussing the association of miRNAs with cancers and ARD. Each entry in miRACA contains the information on miRNAs, the associated disease, miRNA target gene, p-value assigned to the association, and the literature reference. Further, information about the miRNA sequences, its chromosome number and location has also been included.

A user-friendly web interface was developed for the ease of data retrieval, and the application server Apache was used for the purpose. The presentation layer of miRACA was created using XHTML and CSS, while for the backend database, MySQL was used. The programming language used was PHP.

Results

miRNAs associated with ARD and cancers

The collection of miRNAs associated with ARD consisted of 4169 entries while 7893 entries were found for miRNAs associated with cancers. The phenotypes considered in ARD and cancers are included in Tables 1 and 2 respectively.

miRNA-disease network analysis

A network was constructed to establish miRNA-disease association in both ARD and cancers. To construct the miRNA-disease bipartite network, the data of miRNAs associated with ARD and cancers extracted were used to derive two networks, namely, miRNA-ARD network and miRNA-cancers network which displayed a substantial interaction pattern between ARD and cancers.

In Fig. 2, Breast neoplasm with the highest degree of 243 was found to be the hub disease in miRNA-ARD bipartite network with closeness centrality value of 0.52 which was followed by stomach neoplasm (degree-205, closeness centrality value-0.47) and colorectal neoplasms (degree-170, closeness centrality value-0.45). Of all the miRNAs, hsa-mir-21 with the highest degree of 30 was found to be the hub miRNA associated with the majority of ARDs with a closeness centrality value of 0.47 which was followed by hsa-mir-155 (degree-24, closeness centrality value-0.46 and hsa-mir-126 (degree-23, closeness centrality value-0.46).

In miRNA-cancer bipartite network analysis (Fig. 3), hepatocellular carcinoma with the highest degree of 249 was found to be the hub cancer with a closeness centrality value of 0.40. Hepatocellular carcinoma was followed by breast neoplasm with the second highest degree of 243 (closeness centrality value-0.48) and stomach neoplasms (degree-205, closeness centrality value-0.45). Of all the miRNAs, hsa-mir-21 with the highest degree of 59 was again found to be the

Table 1 Phenotypes included in ARD data collection

Colonic neoplasms	Coronary artery disease
Lung neoplasms	Ovarian neoplasms
Breast neoplasms	Urinary bladder neoplasms
Pancreatic neoplasms	Long qt syndrome
Prostatic neoplasms	Parkinson's disease
Schizophrenia	Colorectal neoplasms
Stomach neoplasms	Obesity
Cardiac arrhythmias	Uterine cervical neoplasms
Diabetes mellitus	Graves' disease
Liver neoplasms	Basal cell carcinoma
Glioblastoma	Hyperlipidemias
Alzheimer's disease	Mouth neoplasms
Melanoma	Medulloblastoma
Dementia	Cardiomegaly
Gastrointestinal neoplasms	Gastric neoplasms
Myocardial infarction	Bladder neoplasms
Lymphoma	Prostate neoplasms
Cardiomyopathies	Stomach neoplasms
Carotid artery diseases	Osteosarcoma
Hearing loss	Anxiety disorders
Osteoarthritis	Colon Neoplasms
Type 2 diabetes mellitus	Atrial fibrillation
Neurodegenerative diseases	Diabetic retinopathy
Rheumatoid arthritis	Systemic lupus erythematosus
Hypertension	Osteosarcoma
Arthritis	Mesothelioma
Atherosclerosis	Chronic obstructive pulmonary disease
Adenocarcinoma	Diabetic nephropathies
Colorectal neoplasms, hereditary	Osteoporosis
Brain neoplasms	Type 1 diabetes mellitus
Multiple sclerosis	Neuroblastoma
Acute coronary syndrome	Crohn's disease
Ulcerative colitis	Renal cell carcinoma
Cardiovascular diseases	Squamous cell carcinoma
Pulmonary fibrosis	Squamous cell carcinoma

Table 2 Phenotypes included in cancer data collection

Lung neoplasms	Leukemia
Breast neoplasms	Prostate neoplasms
Hepatocellular carcinoma	Ovarian neoplasms
Pancreatic neoplasms	Uterine cervical neoplasms
Gastric neoplasms	Thyroid neoplasms
Bladder neoplasms	Urinary bladder neoplasms
Stomach neoplasms	Oral carcinoma
Cholangiocarcinoma	Stomach neoplasms
Colorectal neoplasms	Osteosarcoma
Burkitt's lymphoma	Colon neoplasms
Hodgkin's disease	Glioblastoma
Lymphoma	Mesothelioma
Multiple myeloma	Endometrial neoplasms
Pituitary neoplasms	Kidney neoplasms
Hematologic neoplasms	Laryngeal neoplasms
Brain neoplasms	Synovial sarcoma
Leiomyosarcoma	Ewing's sarcoma
Papilary thyroid carcinoma	Squamous cell neoplasms
Neuroblastoma	Hemangiosarcoma
Testicular neoplasms	Basal cell carcinoma
Nasopharyngeal neoplasms	Small cell carcinoma
Multiple hamartoma syndrome	Biliary tract neoplasms
Melanoma	Fibrosarcoma
Liposarcoma	Hypopharyngeal neoplasms
Astrocytoma	Kaposi's sarcoma
Renal cell carcinoma	Medulloblastoma
Adenocarcinoma	Cerebellar neoplasms
Esophageal neoplasms	Salivary gland neoplasms
Choriocarcinoma	Adrenocortical carcinoma
Gastrointestinal neoplasms	Colorectal neoplasms
Squamous cell carcinoma	Retinoblastoma
Nerve sheath neoplasms	Osteosarcoma
Oligodendroglioma	Liver neoplasms
Rectal neoplasms	Adrenocortical adenoma
Rhabdomyosarcoma	Adrenal cortex neoplasms
Waldenstrom macroglobulinemia	Hepatoblastoma

hub miRNA associated with the majority of cancers with a closeness centrality value of 0.48 followed by hsa-mir-125b-1 (degree-44, closeness centrality value-0.46) and hsa-mir-17 (degree-44, closeness centrality value-0.45). Thus, it can be comprehended that hsa-mir-21 is a key player in both ARD and cancers.

Identifying the shared miRNAs and their target genes

The miRNAs involved in both ARD and cancer was used to develop a Venn diagram to identify the shared or common miRNAs that are associated with both the diseases (Fig. 4).

The results demonstrated the involvement of 1648 miRNAs that were common in both ARD and cancer. After identifying the shared miRNAs successfully, these miRNAs were extracted, and their respective target genes were predicted to determine the biological targets of these miRNAs whereby they control gene expression and ultimately regulate the cellular and molecular responses during disease development and progression (Supplementary Table 1).

miRNA-target gene network analysis

A network was constructed to establish miRNA-target gene interaction of the identified shared miRNAs. The data of the shared miRNAs and its respective target genes extracted were used to derive the network. The bipartite network generated displayed a substantial interlinked interaction pattern between ARD and cancers.

In Fig. 5, of all the target genes, BCL2 with the highest degree of 46 was found to be the hub target gene showing highest interaction with a closeness centrality value of 0.30 which was followed by ZEB1 (degree-24, closeness centrality value-0.27) and ITGB3 (degree-23, closeness centrality value-0.26). hsa-mir-31 with the highest degree of 12 was found to be the hub miRNA showing highest interaction with a closeness centrality value of 0.27.

Taking degree value 9 as the threshold value, we selected hsa-mir-221, hsa-mir-21, hsa-mir-12b-1, hsa-mir-34b, hsa-mir-34c, hsa-mir-126, hsa-mir-125b-2, hsa-mir-148a, hsa-mir-143, hsa-mir-16-1 and hsa-let-7g for further analysis (Table 3).

Functional enrichment analysis

To group the similar, redundant, and heterogeneous genes from the entire target gene list for deciphering the biological meaning, functional enrichment analysis was conducted using DAVID (Huang et al., 2009a, 2009b). We performed functional enrichment analysis of the target genes of those selected shared miRNAs that were shown to have maximum interaction. Total 86 unique genes were submitted as a gene list among them DAVID database converted 79 genes as a gene list. These 79 DAVID selected genes were then converted to DAVID gene IDs using Gene Accession Conversion Tool. Based on the enrichment score, the submitted gene list was classified into total 6 clusters considering the stringency to be highest (Supplementary Table 2). The first cluster had the highest enrichment score of 2.21, and where the miRNA target genes were found to be involved in three main gene classes, i.e., small GTPase superfamily, prenylation gene family, and small GTP binding protein domain family (Table 4).

Ontology analysis

We performed ontology analysis of the target genes of those selected shared miRNAs that were shown to have maximum interaction. Total 86 unique genes were submitted as gene list among which the database identified 79 genes. Hence, the results show the distribution of 79 genes along with the three

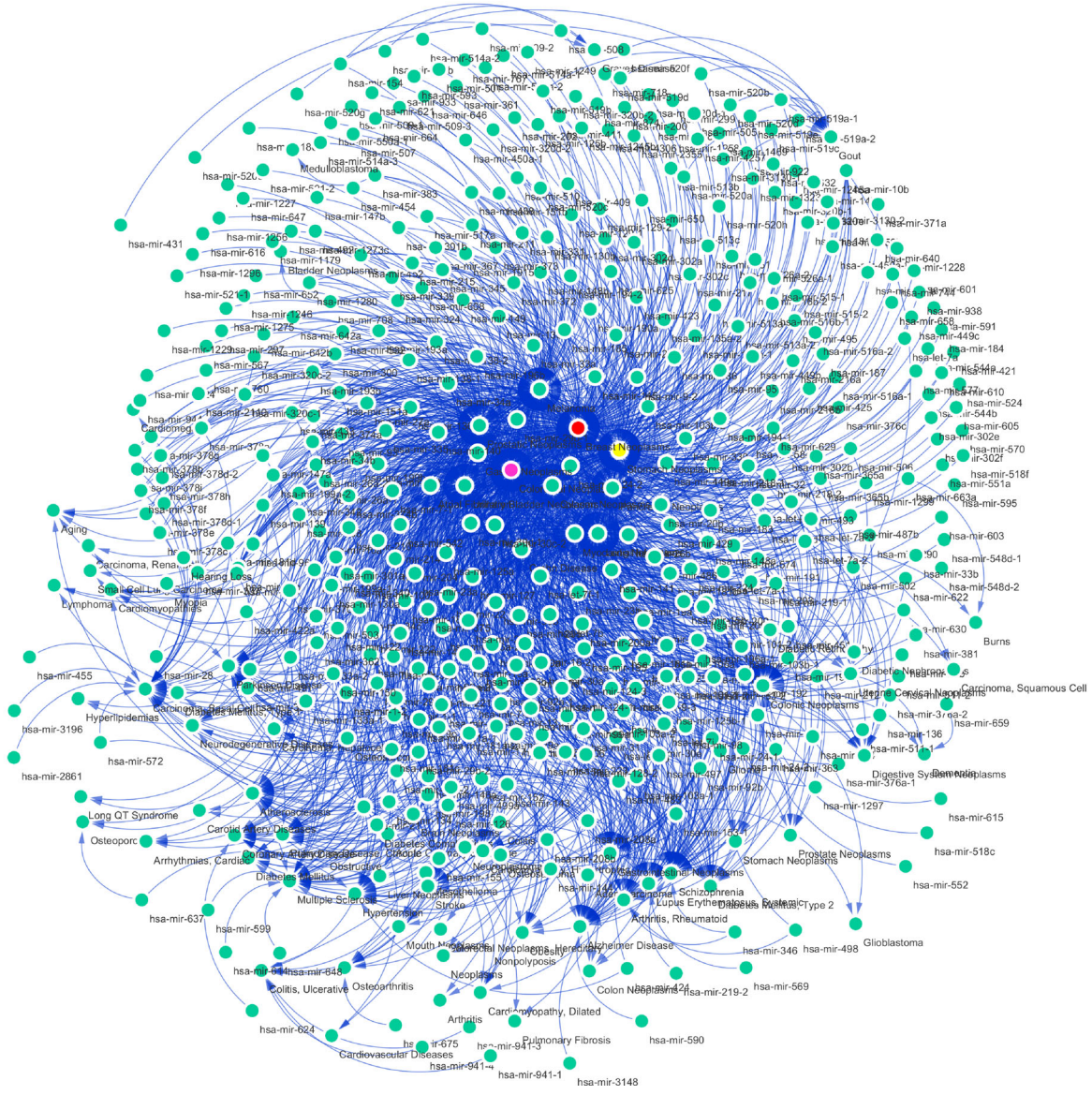


Figure 2 miRNA-ARD bipartite network. The red colored node represents breast neoplasm, yellow colored node represents stomach neoplasm, and pink colored node represents colorectal neoplasm.

aspects of ontology which were considered, namely, biological process, molecular function and pathway (Fig. 6).

Biological process analysis of miRNA target genes

In our biological process analysis, the miRNA target genes were also shown to be majorly involved in the cellular process and metabolic process. Total 86 unique miRNA target genes were submitted as gene list among which the database identified 79 genes as a match to their database. Out of the total of 86 genes (Fig. 6A), 54 genes (62.8%) were shown to be involved in the cellular process, and 38 genes (44.2%) were shown to be involved in the metabolic process (Table 5).

Molecular function analysis of miRNA target genes

We also conducted the molecular function analysis of the target genes of the selected miRNAs which showed them to be majorly involved in binding and catalytic activity.

Out of the total of 86 genes (Fig. 6B), 44 genes (51.2%) were shown to be involved in binding, and 32 (37.2%) genes were shown to be involved in catalytic activity (Table 6).

Pathway analysis of miRNA target genes

Pathway analysis of the target genes of the selected miRNAs showed to be primarily involved in CCKR (cholecystokinin receptor) signaling map and inflammation mediated by chemokine and cytokine signaling pathway.

Out of the total of 86 genes (Fig. 6C), 12 genes (14%) were shown to be involved in CCKR signaling map and inflammation mediated by chemokine and cytokine signaling pathway respectively (Table 7).

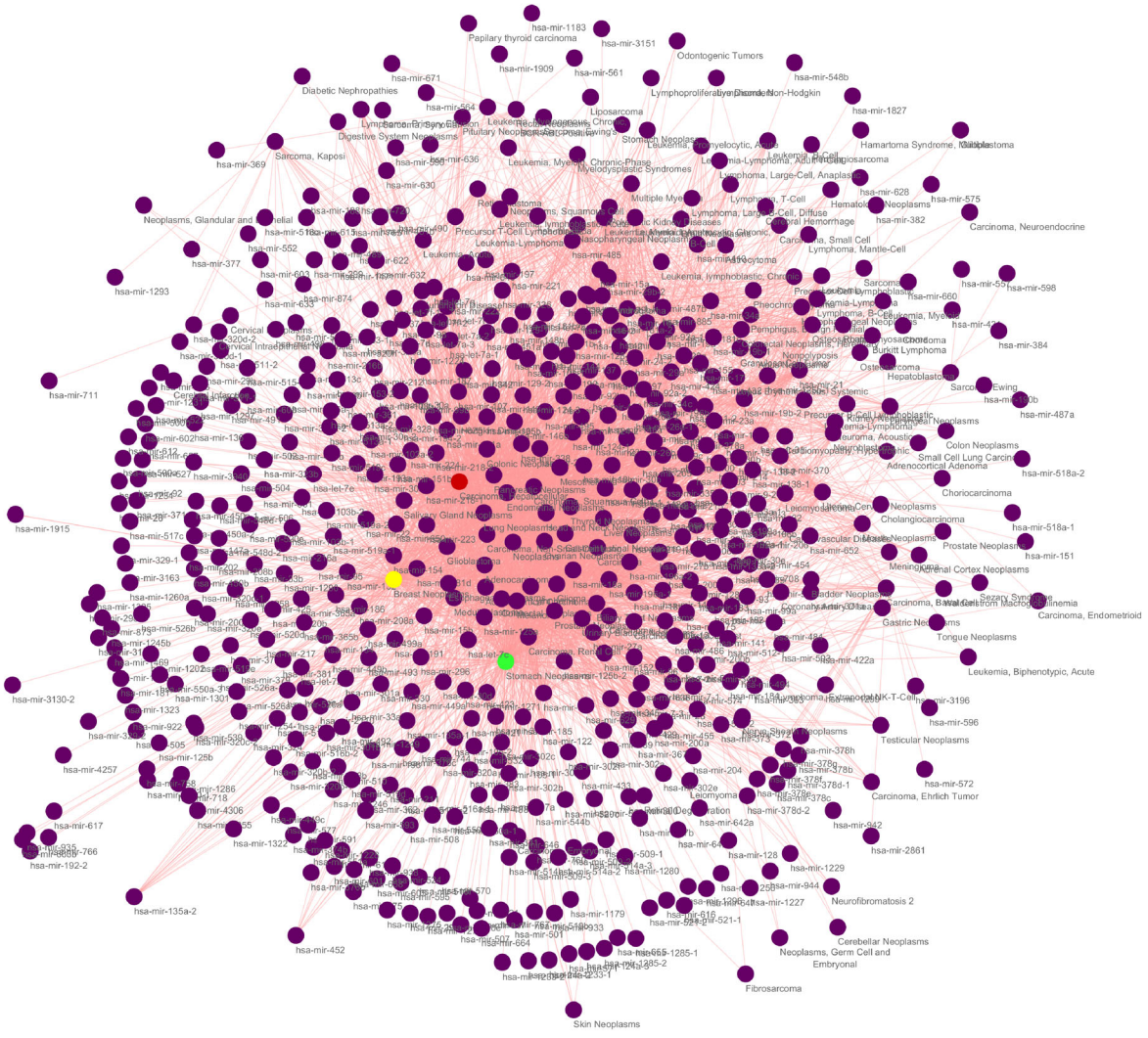


Figure 3 miRNA-cancer bipartite network. The red colored node represents hepatocellular carcinoma, yellow colored node represents breast neoplasm, and green colored node represents stomach neoplasm.

miRACA

miRACA is a manually curated database with an aim to provide a freely accessible source of information about the association of shared miRNAs in cancers and ARD. It attempts to facilitate access to, and the analysis of the relationships asserted between miRNAs and the observed disease conditions. Currently, miRACA has information on 1648 miRNAs associated with 38 diseases under 18 classes. The distribution of the shared (common) miRNAs in the various classes of diseases has been represented in Fig. 7. Table 8 lists all the phenotypes included in miRACA.

miRACA provides a user-friendly interface to detailed information on each miRNA-disease association (Fig. 8A). The users can search their query in the database and access information through miRNA, its target gene or disease (phenotype) (Fig. 8B, 8C).

The database can be queried individually or in combination with any of the categories as mentioned above. Further, the database interface allows for the selection of the attributes,

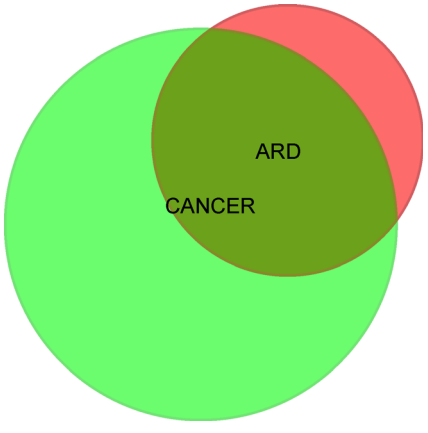


Figure 4 Shared miRNAs asscociated with both ARD and cancers.

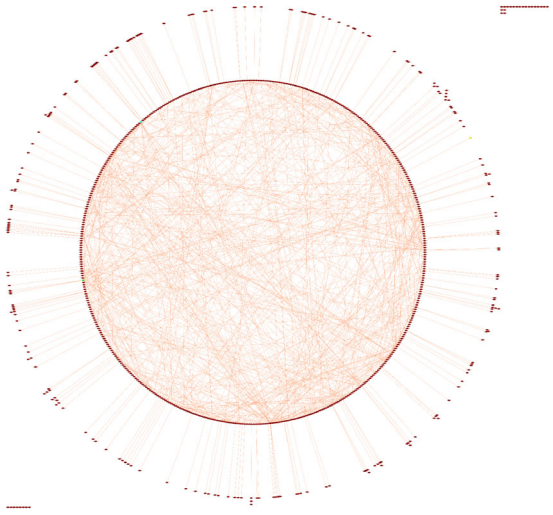


Figure 5 miRNA-target gene bipartite network. The yellow colored node represents BCL2, green colored node represents ZEB1, and blue colored node represents ITGB3.

Table 3 Degree and closeness centrality values of the selected shared miRNAs (taking degree value 9 as the threshold value)

miRNA	Closeness centrality value	Degree
hsa-mir-31	0.27	12
hsa-mir-221	0.25	11
hsa-mir-21	0.24	11
hsa-mir-125b-1	0.27	10
hsa-mir-34b	0.26	10
hsa-mir-34c	0.24	10
hsa-mir-126	0.24	10
hsa-mir-125b-2	0.26	9
hsa-mir-148a	0.26	9
hsa-mir-143	0.26	9
hsa-mir-16-1	0.25	9
hsa-let-7g	0.25	9

Table 4 Functional annotation clustering analysis of the selected shared miRNA target genes with the highest enrichment score (data shown for first cluster only)

Cluster	Enrichment score-2.21	*p*-value
1	Small GTPase superfamily	0.002
	Prenylation	0.005
	Small GTP binding protein domain	0.008
	GTP binding	0.002

such as PubMed ID, miRNA sequences, chromosome number, chromosome location and p-value. This information may, therefore, be used for various associative studies relating to miRNAs to uncover their role and importance in disease susceptibility and pathogenesis.

Table 5 Biological process analysis of the selected shared miRNA target genes

Biological processes	Number of genes (percentage)
Cellular processes	54 genes (62.8%)
Metabolic process	38 genes (44.2%)
Developmental process	27 genes (31.4%)
Response to stimulus	24 genes (27.9%)
Biological regulation	16 genes (18.6%)
Immune system process	11 genes (12.8%)
Multicellular organismal process	9 genes (10.5%)
Cellular component organization or biogenesis	7 genes (8.1%)
Biological adhesion	6 genes (7%)
Reproduction	2 genes (2.3%)
Localization	2 genes (2.3%)
Locomotion	2 genes (2.3%)

Table 6 Molecular function analysis of the selected shared miRNA target genes

Molecular functions	Number of genes (percentage)
Binding	44 genes (51.2%)
Catalytic activity	32 genes (37.2%)
Receptor activity	5 genes (5.8%)
Structural molecule activity	2 genes (2.3%)
Translation regulator activity	1 gene (1.2%)
Transporter activity	1 gene (1.2%)
Signal transducer activity	1 gene (1.2%)

Discussion

As established in several studies, it is not surprising given the involvement of miRNAs in the regulation of multiple cellular processes that miRNAs play an essential part in the regulation of genes related to aging as well as human disease mechanisms. The notable role of miRNAs in mediating complex interlinked pathways evinces that the attributes that designate the aging process may be brought about by the action of an integrated and coordinated pool of miRNAs (Jung and Suh, 2012). The labyrinthine changes that occur in human and other species and the relative influence of individual miRNAs within will probably be disparate from tissue to tissue and organism to organism (Ro et al., 2007).

A number of miRNA studies conducted identified miRNAs which modulate the aging process and whose involvement regulates cancer development and proliferation. Approximately 200 miRNAs have been ascertained, in various cancer malignancies, which are deregulated posing as a significant evidence of the involvement of such miRNAs and their implications in cancer through targeting proto-oncogenes or tumor suppressor genes (Palmero et al., 2011). However, the fundamental contribution of the interlinked nature of

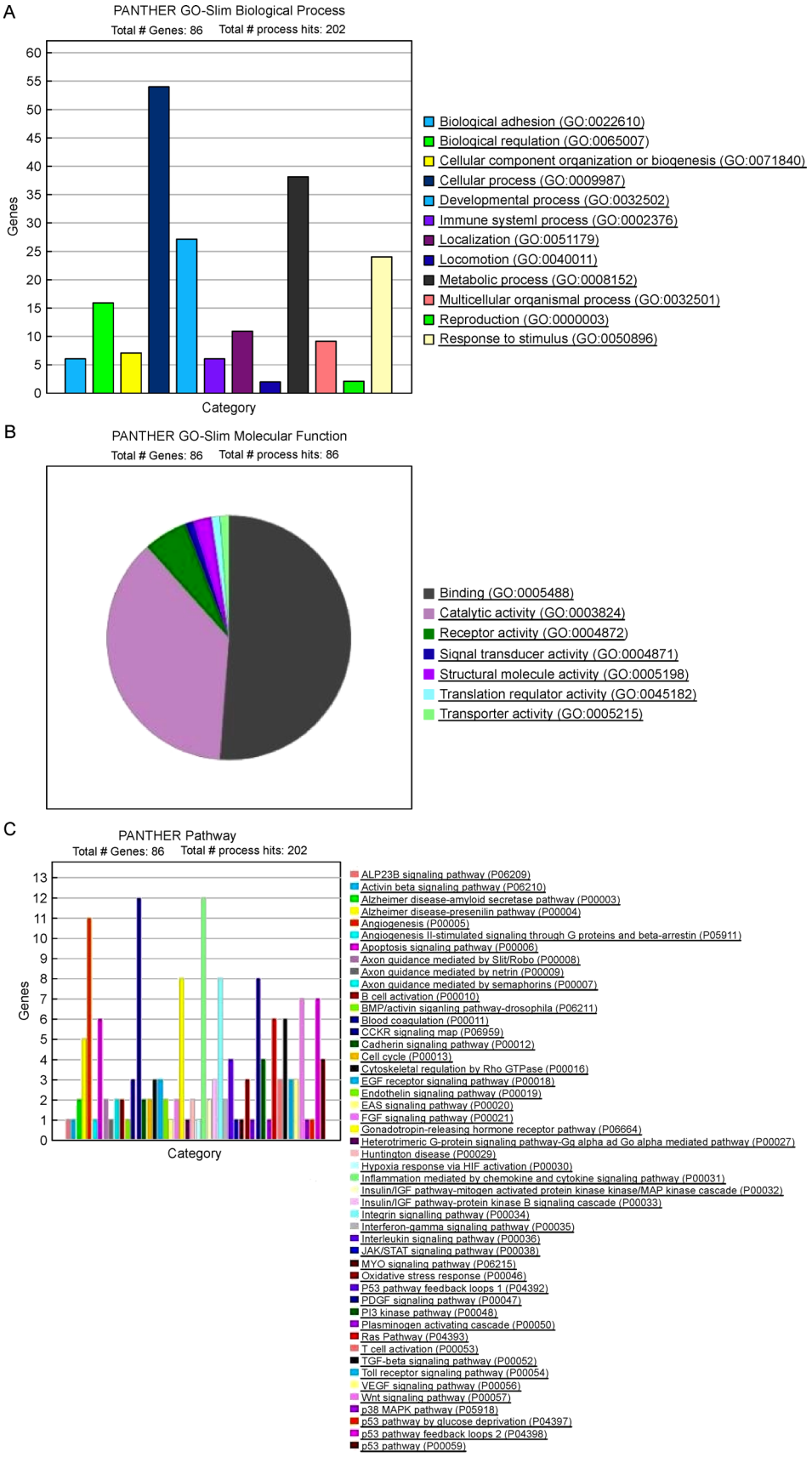


Figure 6 (A) Biological process analysis of the selected shared miRNA target genes. (B) Molecular function analysis of the selected shared miRNA target genes. (C) Pathway analysis of the selected shared miRNA target genes.

Table 7 Pathway analysis of the selected shared miRNA target genes

Pathways	Number of genes (percentage)
CCKR (cholecystokinin receptor) signaling map	12 genes (14%)
Inflammation mediated by chemokine and cytokine signaling pathway	12 genes (14%)
Angiogenesis	11 genes (12.8%)
Gonadotropin-releasing hormone receptor pathway	8 genes (9.3%)
PDGF signaling pathway	8 genes (9.3%)
Integrin signaling pathway	8 genes (9.3%)
Wnt signaling pathway	7 genes (8.1%)
p53 pathway feedback loops 2	7 genes (8.1%)
Apoptosis signaling pathway	6 genes (7%)
Ras Pathway	6 genes (7%)
TGF-beta signaling pathway	6 genes (7%)
Alzheimer disease-presenilin pathway	5 genes (5.8%)
Interleukin signaling pathway	4 genes (4.7%)
pI3 kinase pathway	4 genes (4.7%)
p53 pathway	4 genes (4.7%)
T cell activation	3 genes (3.5%)
Oxidative stress response	3 genes (3.5%)
Toll receptor signaling pathway	3 genes (3.5%)
VEGF signaling pathway	3 genes (3.5%)
Insulin/IGF pathway-protein kinase B signaling cascade	3 genes (3.5%)
Cytoskeletal regulation by Rho GTPase	3 genes (3.5%)
EGF receptor signaling pathway	3 genes (3.5%)
Blood coagulation	3 genes (3.5%)
Alzheimer disease-amyloid secretase pathway	2 genes (2.3%)
axon guidance mediated by semaphorins	2 genes (2.3%)
B cell activation	2 genes (2.3%)
Axon guidance mediated by Slit/Robo	2 genes (2.3%)
Cadherin signaling pathway	2 genes (2.3%)
Cell cycle	2 genes (2.3%)
Endothelin signaling pathway	2 genes (2.3%)
Interferon-gamma signaling pathway	2 genes (2.3%)
FGF signaling pathway	2 genes (2.3%)
Huntington disease	2 genes (2.3%)
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	2 genes (2.3%)
FAS signaling pathway	1 gene (1.2%)
p38 MAPK pathway	1 gene (1.2%)
p53 pathway by glucose deprivation	1 gene (1.2%)
Plasminogen activating cascade	1 gene (1.2%)
JAK/STAT signaling pathway	1 gene (1.2%)
MYO signaling pathway	1 gene (1.2%)
Hypoxia response via HIF activation	1 gene (1.2%)
p53 pathway feedback loops 1	1 gene (1.2%)
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	1 gene (1.2%)
Axon guidance mediated by netrin	1 gene (1.2%)
ALP23B signaling pathway	1 gene (1.2%)
Activin beta signaling pathway	1 gene (1.2%)
BMP/activin signaling pathway-drosophila	1 gene (1.2%)
Angiotensin ii-stimulated signaling through g proteins and beta-arrestin	1 gene (1.2%)

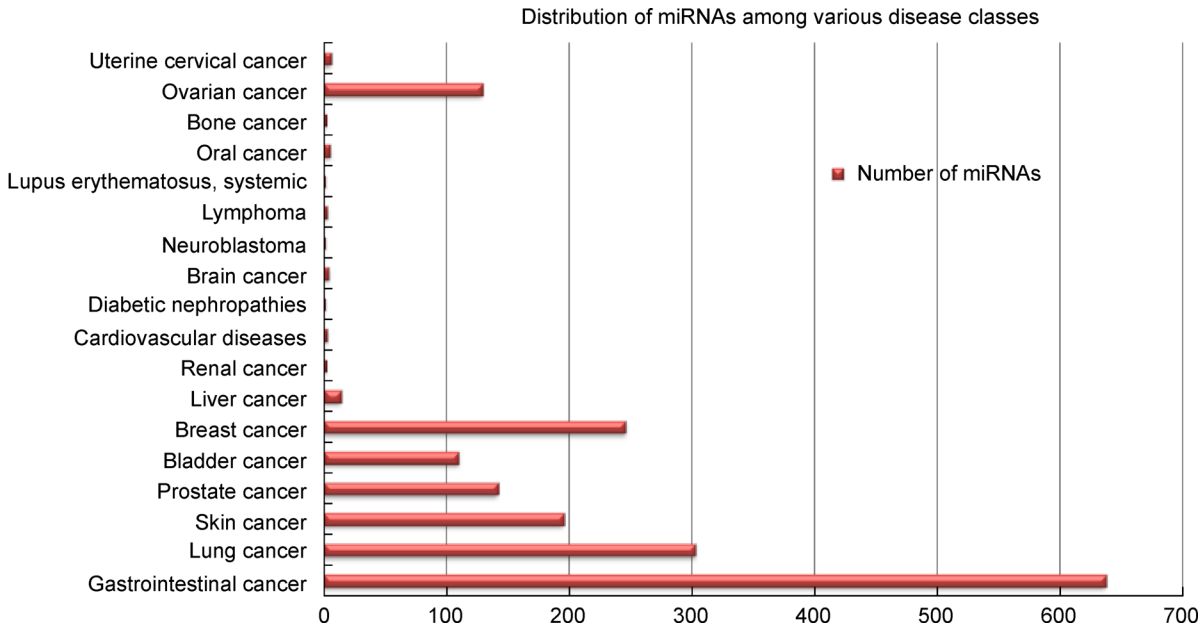


Figure 7 Graph showing the distribution of disease class in miRACA.

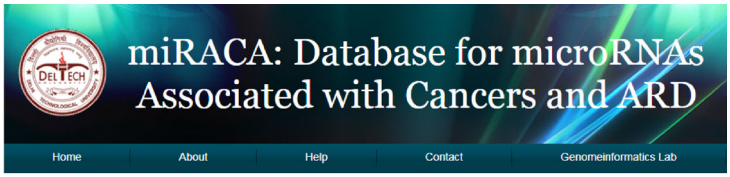
Table 8 List of disease class included in miRACA

S. No.	Disease class
1	Gastrointestinal cancer
2	Lung cancer
3	Skin cancer
4	Prostate cancer
5	Bladder cancer
6	Breast cancer
7	Liver cancer
8	Renal cancer
9	Cardiovascular diseases
10	Diabetic nephropathies
11	Brain cancer
12	Neuroblastoma
13	Lymphoma
14	Lupus erythematosus, systemic
15	Oral cancer
16	Bone cancer
17	Ovarian cancer
18	Uterine cervical cancer

miRNAs associated with aging and cancer is not yet completely understood.

To discover interaction of miRNAs with ARD and cancer, miRNAs associated specifically with these diseases were characterized. The identification and characterization of the miRNA target genes related to ARD and cancers have a practical and heuristic relevance as it regulates several cellular and molecular processes.

Our present study acknowledged 436 miRNAs associated with ARD and 515 miRNAs associated with cancers. Identification of shared miRNAs associated with both ARD and cancers resulted in 415 out of 951 miRNAs. Our network analysis results revealed a few essential nodes (called hubs) that show maximum interaction to a large number of neighboring nodes in the network implying the significant association of miRNAs and their target genes with disease pathogenesis concerning both cancers and ARD. Our miRNA-disease association analysis results showed breast neoplasm and hepatocellular carcinoma more pronounced to be regulated by miRNAs which supports the results of related studies (Gramantieri et al., 2008; Kalyani et al., 2011; Mulrane et al., 2013; Serpico et al., 2014; Chang-Hao et al., 2015; Takahashi et al., 2015). Also, hsa-mir-21 and hsa-mir-31 were found to play a significant role in the development and progression of cancer as well as ARD. This signifies the potentiality of miRNAs to regulated breast cancer and hepatocellular carcinoma as established in recent studies (Dellago et al., 2013). Furthermore, BCL2 was observed as the hub target gene as it exhibited interactions with several miRNAs in the network (Kang and Pervaiz, 2013; Yip and Reed, 2008). The magnitude of change caused as a result of knocking out or blocking such fundamental hubs dictates the relative importance of the particular hub node in the network. As such, the inhibition of such hub miRNAs or target genes may be lethal for the network according to the centrality-lethality rule, as it would attenuate their effect on disease development and progression (He and Zhang, 2006). Therefore, our analysis indicates that both hsa-mir-21 and hsa-mir-31 can be a prime target for further investigative studies to dissect their prospective role in strategic treatment approaches. In addition, BCL2 can be targeted to regulate the related specific miRNAs which in turn can modulate the onset and development of the multiple associated diseases.

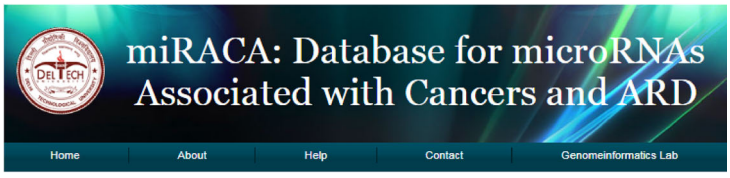


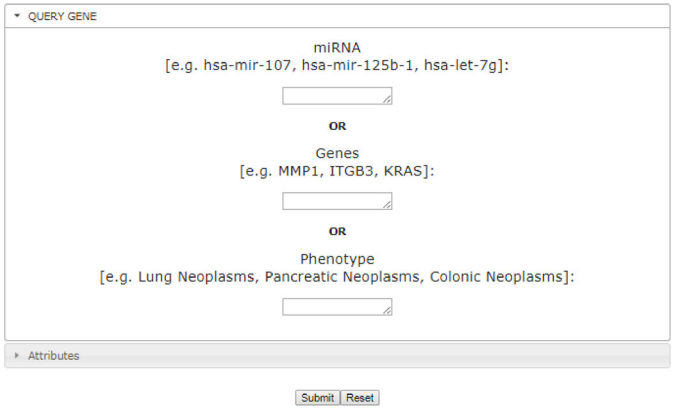
Welcome to miRACA

With the given diversity and abundance of several targets of miRNAs, they functionally appear to interact with diverse elements of the multiple cellular networks such as cell growth and development, differentiation, proliferation, and apoptosis. They thereby function either as tumor suppressors or oncogenes, whose under or overexpression plays a significant role in the development and progression of several cancers such as leukemia, liver, lung, pancreatic, colorectal and ovarian cancer which have also been established to be Age Related Disorders (ARD). Since miRNAs have been implicated as prospective regulators of age-related diseases or disorders, the interlinking of miRNAs and longevity can demonstrate the role of miRNAs in the regulation of the aging process and its related disorders.

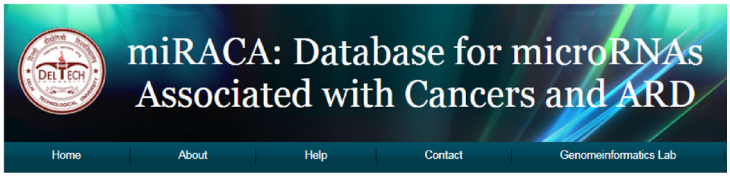
We have, thus, comprehensively curated all miRNAs reported to be associated with both ARD (Age Related Disorders) and cancers in the human genome and established a database that stores all miRNAs related to both the diseases along with its target genes. The database currently houses 1648 miRNAs associated with both cancers and ARD comprising of 38 variants supported with pertinent data. Also, the database includes the corresponding miRNA sequences, its chromosome number, and location.

A





B



miRNA	Phenotype	Genes	Pubmed ID	Chromosome number	Chromosome location	p-value
hsa-mir-107	Colonic Neoplasms	DAPK	16461460	chr10	10q23.31	<0.05
hsa-mir-107	Stomach Neoplasms	CDK6	22407237	chr10	10q23.31	<0.05
hsa-mir-107	Breast Neoplasms	SDC1	16461460	chr10	10q23.31	<0.05
hsa-mir-107	Lung Neoplasms	ID1	16461460	chr10	10q23.31	<0.05
hsa-mir-107	Melanoma	JUN	16461460	chr10	10q23.31	<0.05
hsa-mir-1260	Melanoma					
hsa-mir-107	Gastrointestinal Neoplasms	CDK6	16461460	chr10	10q23.31	<0.05
hsa-mir-107	Urinary Bladder Neoplasms	DAPK	16461460	chr10	10q23.31	<0.05
hsa-mir-107	Prostatic Neoplasms	PBX3	22240788	chr10	10q23.31	<0.05
hsa-mir-1260	Colorectal Neoplasms					
hsa-mir-107	Colorectal Neoplasms	DAPK	22593189	chr10	10q23.31	<0.05
hsa-mir-1260	Urinary Bladder Neoplasms					

C

Figure 8 miRACA database. (A) Introduction page. (B) Home (Query) page. (C) Results page.

Our functional enrichment analysis of the miRNA target genes revealed that the majority of the target genes belonged to GTPase superfamily (He et al., 2008). The gene ontology enrichment analysis explicitly showed that the miRNA target genes were involved in the cellular and metabolic process and their molecular functions which include binding and catalytic activity. Also, the gene ontology pathway analysis divulges CCKR (cholecystokinin receptor) signaling map (cancer), gonadotropin-releasing hormone receptor pathway (growth), inflammation mediated by chemokine and cytokine signaling pathway (cancer) as a significant metabolic pathway for miRNA target genes. CCKR signaling pathway plays a prime role in the control of cell proliferation in cancer cells and mediates the proliferative responses induced by the hormonal GI peptides in normal as well as cancer cells (Rozengurt and Walsh, 2001). Also, Bcl-2 protein family specifically modulates CCKR signaling. Members of the GTPase superfamily are differentially activated by gastrins which in turn regulate various proteins of the Bcl-2 protein family that changes caspase activity, thereby, modulating apoptosis in cancer cells (He and Baldwin, 2008; He et al., 2008). Gonadotropin-releasing hormone receptor pathway has a significant regulatory role in aging and cell fate regulation (Wang et al., 2010). Inflammation mediated by chemokine and cytokine signaling pathway is reported to promote malignant transformation of cells and carcinogenesis (Landskron et al., 2014) and also has an essential contribution in immune senescence (Ponappan S and Ponappan U, 2011). Exploring these pathways may assist in unraveling potential molecular determinants which might foment the development of novel regulatory agents to ameliorate disease conditions. Thus, our computational evaluation of the association of shared miRNAs between cancers and ARD justifies a rationale for further experimental studies to decipher and ascertain the possibility of enhanced prognostic and treatment modalities to mitigate the jeopardy of life in patients and susceptible individuals.

Conclusion

Computational analysis of miRNA networks and its target genes emphasize the role of miRNAs in regulating cancer as well as aging and may open up new avenues for the interpretation and acknowledgment of their association with aging and disease etiology thus improving our understanding of potential diagnostic and treatment targets. We have developed a database miRACA, a user-interactive online repository of miRNAs associated with cancers and ARD. It is made freely available to users at http://genomeinformatics.dtu.ac.in/miraca/.

Mining the database for biologically meaningful information may prove to be helpful to elucidate the underlying modus operandi and close acquaintance between cancer and aging. Data from miRACA may be used to construct networks of miRNA-disease as well as miRNA-target gene associations so as to ease the process of identification of miRNAs and genes significantly involved in disease etiology. Network-based studies of miRNA-disease relationships may provide a deeper insight into how various diseases are associated together. Also, it may help to decipher common miRNAs and pathways which are shared by a class of diseases. We believe that exploring the role of miRNAs would be helpful in predicting which of them confer susceptibility to various diseases, thereby, providing a means for better drug repositioning and further progress in the field of personalized medicine. Thus, the present work may be useful to unveil shrouded links between cancer and age-related disorders providing valuable perspective to physicians, counselors, and biomedical researchers.

Abbreviations

ARD: Age related disorders; CCKR: Cholecystokinin receptor; dbAARD: database of Aging and Age Related Disorders; DAVID: The Database for Annotation, Visualization and Integrated Discovery; GO: Gene Ontology; HMDD: the Human microRNA Disease Database; miRACA: Database for microRNAs Associated with Cancers and ARD; PANTHER: Protein Analysis Through Evolutionary Relationships

Compliance with ethics guidelines

Razia Rahman, Lokesh Kumar Gahlot and Yasha Hasija declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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