The analysis of miRNA expression profiling datasets reveals inverse microRNA patterns in glioblastoma and Alzheimer's disease

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Abstract. There is recent evidence to indicate the existence of an inverse association between the incidence of neurological disorders and cancer development. Concurrently, the transcriptional pathways responsible for the onset of glioblastoma multiforme (GBM) and Alzheimer's disease (AD) have been found to be mutually exclusive between the two pathologies. Despite advancements being made concerning the knowledge of the molecular mechanisms responsible for the development of GBM and AD, little is known about the identity of the microRNA (miRNAs or miRs) involved in the development and progression of these two pathologies and their possible inverse expression patterns. On these bases, the aim of the present study was to identify a set of miRNAs significantly de-regulated in both GBM and AD, and hence to determine whether the identified miRNAs exhibit an inverse association within the two pathologies. For this purpose, miRNA expression profiling datasets derived from the Gene Expression Omnibus (GEO) DataSets and relative to GBM and AD were used. Once the miRNAs significantly de-regulated in both pathologies were identified, DIANA-mirPath pathway prediction and STRING Gene Ontology enrichment analyses were performed to establish their functional roles in each of the pathologies. The results allowed the identification of a set of miRNAs found de-regulated in both GBM and AD, whose expression levels were inversely associated in the two pathologies. In particular, a strong negative association was observed between the expression levels of miRNAs in GBM compared to AD, suggesting that although the molecular pathways behind the development of these two pathologies are the same, they appear to be inversely regulated by miRNAs. Despite the identification of this set of miRNAs which may be used for diagnostic, prognostic and therapeutic purposes, further functional *in vitro* and *in vivo* evaluations are warranted in order to validate the diagnostic and therapeutic potential of the identified miRNAs, as well as their involvement in the development of GBM and AD.

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Introduction

Over the past century, human life expectancy has increased significantly, settling at approximately 76.3 years for males and 81.2 for females (1). Such an increment in lifespan has been associated with a huge surge in the most diffused life-threatening diseases, including cardiovascular, neurodegenerative and oncological pathologies (2).

Although aging represents a common risk factor, the physiological and molecular mechanisms behind the development of age-related disorders differ completely, particularly when comparing cancer with neurodegenerative disorders (3,4). Supporting this evidence, recent epidemiological data have demonstrated that there is an inverse association between cancer and neurodegeneration (5,6).

Despite this inverse association between the incidence of tumors and the rise of neurodegenerative disorders, some conditions, such as inflammation (7-9), the alteration of the intestinal microbiota (10-13), diet-related diseases (14-18) and risks related to the exposure to environmental pollutants (19-25) are involved in the development of both pathologies. Consistently, the association between the development of neurodegenerative diseases and the onset of tumors may be either direct or indirect. As a direct consequence, it has been shown that several benign and malignant types of cancer are associated with neurological, as well as neurodegenerative disorders (26-28).

In particular, it has been demonstrated that although glioblastoma multiforme (GBM) and Alzheimer's disease (AD) share the same molecular pathways, substantial differences exist in their modulation (29,30). In fact, while rapid cell proliferation and apoptotic cell arrest are typical features of GBM, cellular damage and subsequent cell death are common consequences in AD (31,32).

To date, the only available treatments for AD are only palliative, as they are capable of delaying memory and cognitive function impairment, without actually blocking neuronal loss. The main drugs used in the treatment of AD are cholinesterase inhibitors and glutamatergic N-methyl D-aspartate (NMDA) receptors antagonists, such as memantine (Namenda), respectively able to improve neuropsychiatric symptoms and neuronal cell-to-cell communication (33). Importantly, the benefits of such therapies are not long-lasting and are coupled with adverse impairing effects.

GBM is a widely diffused brain malignancy, as well as the most aggressive tumor of the central nervous system (34,35). Currently, GBM treatment options are limited, typically represented by surgical resection of the tumor mass (when the lesion does not involve vascular and nerve structures), followed by radiotherapy and chemotherapy (36). Despite advancements being made in anticancer treatments (37), the therapeutic approaches available for GBM are often ineffective, given the high rate of GBM relapse and drug resistance (36). Recently, *in vitro* studies have demonstrated that treatment with nitric oxide-releasing HIV protease inhibitors previously adopted for other tumors (38,39), is effective in reducing the proliferation of GBM cancer cells (40,41).

Notably, the diagnostic and therapeutic approaches that are normally used to recognize and to treat cognitive deficits and dementia symptoms, appear to be slightly effective in the early detection and treatment of brain precancerous lesions. Although they need to be further validated in a larger cohort of patients, transcranial magnetic stimulation (TMS) and transcranial Doppler ultrasonography, normally used for vascular cognitive impairments (42-47), AD (48), restless leg syndrome (49-52), and other neurological syndromes (53-56), appear to be promising approaches suitable for the diagnosis and cure of precancerous brain lesions.

A concern about GBM management is relative to the lack of effective biomarkers. In particular, a number of biomarkers have been proposed to solve this issue. Several studies have highlighted the possible application of extracellular protein biomarkers, such as extracellular matrix proteins, vascular endothelial growth factor (VEGF), angiogenesis-associated proteins, matrix metalloproteinases (MMPs; MMP-2, MMP-9) and astrocyte elevated gene-1 (AEG-1), macrophage migration inhibitory factor (MIF) and functionally-related genes (DD-T; CD74, CD44, CXCR2 and CXCR4) (57-59). Other proteins can be also used for the treatment or prognostic evaluation of tumor development (60,61).

Given the lack of effective diagnostic strategies, as well as treatments able to effectively cure both GBM and AD, there is an urgent need for the identification of novel diagnostic biomarkers and therapeutic targets for the effective treatment of such pathologies. Moreover, the understanding of the expression patterns of such biomarkers may prove to be useful in order to further demonstrate the existence of an inverse association between GBM and AD. In this context, several studies have demonstrated that the evaluation of microRNA (miRNA or miR) expression levels in patients compared with their healthy controls, may provide information on the development of several diseases, including cancer and neurodegenerative disorders (62-65). Indeed, miRNAs are involved in both physiological and pathological processes; therefore studying their alterations in GBM and AD may prove to be helpful in detecting early the onset of such pathologies.

On this ground, the aim of this study was to analyze miRNA expression profiling datasets of GBM and AD obtained from the Gene Expression Omnibus (GEO) DataSets portal in order to identify specific miRNAs de-regulated in both diseases. Once the presence of altered miRNAs shared between GBM and AD would be established, the second aim of this study was to determine whether their expression levels are inversely associated.

Materials and methods

Selection and analysis of GBM and AD miRNA profiling datasets. The selection of both GBM and AD miRNA expression profiling datasets was performed using the publicly available GEO DataSets database, as previously reported (66,67). Briefly, for the selection of GBM miRNA datasets the following search terms were used: '{['non coding rna profiling by array'(DataSet Type)] AND glioblastoma} AND '*Homo sapiens*'[porgn:__txid9606]'; while for the selection of AD datasets the search terms used were as follows: '{['non coding rna profiling by array'(DataSet Type)] AND Alzheimer} AND '*Homo sapiens*'[porgn:__txid9606]'. These search criteria allowed the identification of different miRNA expression datasets of which only those with >10 samples (total of normal and pathological samples) were selected for the further computational analyses. Given the low number of AD miRNA microarray platforms, datasets containing <10 samples were considered. Datasets including the expression data of GBM or AD within *in vitro* models were not considered for the analyses.

Following dataset selection, the data matrices were downloaded and differential analyses were performed between normal and pathological samples using the GEO2R tool available on GEO DataSets. Since different miRNA microarray platforms were adopted, the differentially expressed miRNAs of each dataset were annotated using the last version of miRBase (miRBase version 22) (68). The miRNA expression fold change (FC) was expressed as base-2 logarithm of FC (log2FC) to normalize the miRNA expression values obtained from different microarray platforms.

All the miRNAs with a value of $P \leq 0.01$ were considered for the merging analyses and the following identification of miRNAs involved in GBM and AD.

Identification of miRNAs potentially involved in GBM and AD. The lists of differentially expressed miRNAs obtained from the GBM and AD datasets were merged through a Venn diagram calculating tool, in order to obtain miRNAs shared with >1 dataset (http://bioinformatics.psb.ugent.be/webtools/Venn/). In particular, only the miRNAs contained at least in the 50% of GBM and AD datasets were considered.

The log2FC levels of each miRNA are reported in a graphic table indicating the level of upregulation and downregulation, using red boxes and blue boxes, respectively with different gradient.

Schemes of de-regulated miRNAs were generated for the GBM datasets, AD datasets and for the GBM and AD datasets together, in order to establish the existence of an inverse association between the expression levels of GBM- and AD-related miRNAs.

Involvement of GBM- and AD-related miRNAs in the modulation of glioma- and AD-related pathways. To establish the involvement of the identified miRNAs in the modulation of glioma- and AD-related pathways and their relative target genes, a pathway prediction analysis was performed using the prediction tool DIANA-mirPath version 3 (69), as previously described (70). The DIANA-mirPath analysis was performed comparing the identified miRNAs with the panel of miRNAs involved in both glioma- (hsa05214) and AD-related (hsa05010) pathways.

Gene Ontology (GO) and roles of GBM and AD miRNA-target genes. The GO analysis and the functional roles of the miRNAs-targeted genes related to GBM and AD were evaluated using the enrichment software 'STRING: Functional protein association networks' (https://string-db.org/) (71). In particular, for the genes related to GBM and AD the 'Biological process', 'Molecular function' and 'Cellular component' were determined. Furthermore, the interaction network between genes was determined for both GBM and AD. The STRING analyses were performed for the 13 selected miRNAs resulting from the comparison of GBM and AD miRNA expression profiling datasets.

Statistical analyses. The GEO2R software already normalized the miRNA expression data derived from GEO DataSets. Only miRNAs with a value of $P \leq 0.01$ were considered for further analyses. Furthermore, the GEO2R software automatically calculated the GEO DataSets data P-values. The P-values obtained from the prediction pathway analysis were already calculated using DIANA-mirPath software (V.3.0).

Results

miRNA profiling dataset selection for GBM and AD. The research of miRNA expression profiling datasets performed with GEO2R using specific search terms allowed for the identification of 51 and 9 miRNA profiling datasets for GBM and AD, respectively. The datasets either relative to *in vitro* studies or built with less than 8 samples, including normal and pathological specimens, were excluded from the analysis. Following this filtering, five datasets for GBM and two datasets for AD, were selected for the study. The information of all selected datasets is reported in Table I.

In particular, for GBM, two datasets were developed by Affymetrix (Affymetrix miRNA Array), two developed by Illumina (Illumina Human MicroRNA expression beadchip) and one developed by Exiqon (Exiqon miRCURY LNA microRNA array, 7th generation). For the AD datasets, one was developed by 3D-Gene (3D-Gene Human miRNA V21_1.0.0) and the other one was a custom platform (USC/XJZ Human 0.9 K miRNA-940-v1.0) (Table I).

Identification of miRNAs involved in the development of GBM and AD. Merging the lists of differentially expressed miRNAs in GBM datasets allowed the identification of a set of miRNAs strictly involved in the development and progression of GBM. In particular, among all miRNAs, 35 were found to be de-regulated with concordant expression levels in at least 3 out of 5 GBM miRNA expression datasets (downregulated or upregulated in all datasets) (Fig. 1A). In particular, 14 miRNAs were upregulated and 21 downregulated. Of these miRNAs, the most upregulated miRNAs were the following: hsa-miR-21, hsa-miR-18a, hsa-miR-19a, hsa-miR-25, hsa-miR-16-1, hsa-miR-106a and hsa-miR-106b; and the most downregulated miRNAs were the following: hsa-miR-128, hsa-miR-129, hsa-miR-7, hsa-miR-873, hsa-miR-218, hsa-miR-139 and hsa-miR-770.

Similarly, following the merging of the 2 AD miRNA lists, 7 miRNAs were uncovered. All of them were related to the development of AD. Among these, 5 were upregulated (hsa-miR-134, hsa-miR-185, hsa-miR-198, hsa-miR-659 and hsa-miR-671) and 2 downregulated (hsa-miR-29c and hsa-miR-494) (Fig. 1B).

By comparing the de-regulated miRNAs in both the GBM and AD datasets, it was observed that the expression levels of 12 miRNAs were inversely associated between GBM and AD. Of these, 3 were upregulated in GBM and downregulated in AD (hsa-miR-106a, hsa-miR-20b and hsa-miR-424) and 9 were downregulated in GBM and upregulated in AD (hsa-miR-1224, hsa-miR-129, hsa-miR-139, hsa-miR-330, hsa-miR-433, hsa-miR-485, hsa-miR-487b, hsa-miR-584 and hsa-miR-885). Additionally, hsa-miR-29c was downregulated in both the GBM and AD datasets, suggesting its involvement in both pathologies (Fig. 2).

Pathway prediction analysis of selected miRNAs. To elucidate the role of the de-regulated miRNAs shared between the GBM and AD datasets, DIANA-mirPath analysis was performed. The analysis revealed that all the 13 selected miRNAs, apart from hsa-miR-433, were involved in the regulation of GBM by modulating the expression levels of 34 different genes (Fig. 3). As regards AD, the pathway prediction analysis revealed that, with the exclusion of hsa-miR-433, hsa-miR-485 and hsa-miR-487b, all the remaining identified miRNAs were able to interact with the AD pathway and to target 51 different genes. Notably, the analysis revealed that the miRNAs, hsa-miR-106a, hsa-miR-424 and hsa-miR-330, were able to modulate the expression levels of MAPK1 in both GBM and AD. In particular, hsa-miR-106a and hsa-miR-424 were able to induce the downregulation and upregulation of MAPK1 in GBM and AD, respectively. This could be explained by the inverse association of miR-106a expression levels in GBM and AD. Similarly, hsa-miR-330 was able to induce the upregulation of MAPK1 in GBM, while in AD, this gene was downregulated. In the same manner, the miRNAs, hsa-miR-424, hsa-miR-885 and hsa-miR-29c, were involved in the regulation of several genes belonging to the family of calmodulins (CALM family). In detail, hsa-miR-424 and hsa-miR-885 exhibited an inverse association in modulating the expression of CALM genes,

Table I. Information pertaining to the selected datasets for glioblastoma and Alzheimer's disease.

Series accession	n. normal	n. cancer	Samples	Platform	Author/(Ref.)	Total samples
Glioblastoma datasets						
GSE90604	7	16	Fresh frozen brain tissues and GBM tumor tissues	GPL21572 [miRNA-4] affymetrix multispecies miRNA-4 array	Gulluoglu *et al*, 2017 (No Ref.)	25
GSE25632	5	82	Normal brain tissues and GBM tumor samples	GPL8179 Illumina Human v2 MicroRNA expression beadchip	(112,113)	87
GSE103229	5	5	Normal brain tissues and GBM tumor samples	GPL18058 Exiqon miRCURY LNA microRNA array, 7th generation	Chun *et al*, 2018 (No Ref.)	10
GSE63319	4	11	Normal brain tissues (epileptic) and GBM tumor samples	GPL16384 [miRNA-3] affymetrix multispecies miRNA-3 array	Sarkar *et al*, 2016 (No Ref.)	18
GSE42657	7	5	Normal brain tissues and GBM tumor samples	GPL8179 Illumina Human v2 MicroRNA expression beadchip	(114)	61
Alzheimer's disease datasets						
GSE120584	288	1021	Serum samples	GPL21263 3D-Gene Human miRNA V21_1.0.0	(115)	1601
GSE16759	4	4	Normal brain tissues and AD tissue samples	GPL8757 USC/XJZ Human 0.9 K miRNA-940-v1.0	(116)	8

GBM, glioblastoma multiforme; AD, Alzheimer's disease.

while hsa-miR-29c was downregulated in both the GBM and AD datasets, thus determining the upregulation of CALM3 in both pathologies (Fig. 3).

In addition, the pathway prediction analysis identified the most targeted genes in GBM as IGF1R (targeted by 6 miRNAs), CCND1, CDKN1A, MDM2 (targeted by 5 miRNAs), AKT3, CDK6, E2F1, MAPK1, PIK3R1, PIK3R3 (targeted by 4 miRNAs). Notably, the mostly targeted gene families were PI3K (targeted by 11 miRNAs), CDK (targeted by 10 miRNAs) and E2F (targeted by 5 miRNAs) (data not shown). As regards the AD pathway, the 13 selected miRNAs were able to target mainly the MAPK1 (targeted by 4 miRNAs), APH1A, APP, GSK3B (targeted by 3 miRNAs), ADAM17, ATP2A2, CALM2 and CALM3 (targeted by 2 miRNAs). Of note, the most altered gene families were the ATP (9 miRNAs), NDUF (8 miRNAs), CALM (5 miRNAs) and ADAM (3 miRNAs) families, strictly involved in the development of AD (data not shown).

GO enrichment analysis of selected miRNAs and target genes. The GO analysis performed by STRING allowed to determine the functional roles of the genes related to GBM and AD and targeted by the selected miRNAs. The STRING analysis performed on GBM miRNA-targeted genes revealed that of the 34 genes, 33 were recognized as proteins. Among these 33 proteins, 30 were directly involved in the glioma pathway (Fig. 4D). The clustering of proteins according to the 'Biological process', 'Molecular function' and 'Cellular component' categories revealed that the majority of proteins were involved in the regulation of cellular processes and metabolic processes (Fig. 4A), in the binding of molecules and in catalytic activities (Fig. 4B). Moreover they belong to intracellular organelle and cytoplasm (Fig. 4C).

Similarly, the STRING analysis performed on the list of miRNA-targeted genes obtained for the AD highlighted that 47 of the 51 identified genes were involved in AD pathway (Fig. 5D). As described for GBM, regarding the 'Biological process' category, the identified genes were involved in the cellular and metabolic processes (metabolism of different compounds), but also in the response to stimuli (Fig. 5A). The AD proteins were also significantly involved in the binding of several molecules and less in the catalytic activity, in contrast

A

miRNA_ID / ID Datasets	Glioblastoma Multiforme Datasets				
	GSE90605	GSE63319	GSE25632	GSE42657	GSE103229
Upregulated miRNAs					
hsa-miR-106a	1.5914	1.0776	0.5187	1.4142	
hsa-miR-106b	1.7427	1.6264	0.6222	0.9125	
hsa-miR-155	0.3384	2.7838	2.3919		
hsa-miR-16-1	0.4201	1.0167	1.6763	1.6960	
hsa-miR-17	1.5506	1.1077	1.5941		
hsa-miR-18a	1.8289	1.3706	1.2203	1.8630	
hsa-miR-19a	1.1975		2.1513	1.7774	2.0627
hsa-miR-19b	1.5367	0.8648	1.1824		
hsa-miR-20b	1.4360	1.1470	0.8598	1.8295	
hsa-miR-21	1.7477	3.2140	2.7614		3.9031
hsa-miR-24-2	2.2562		1.2335	1.1823	
hsa-miR-25	2.8613	1.7722		0.5399	
hsa-miR-424	1.7485		0.5340	1.1126	
hsa-miR-93	1.9640	1.3105		0.3062	
Downregulated miRNAs					
hsa-miR-1224	-1.1855		-3.7882	-3.5978	
hsa-miR-124	-2.6202	-3.3673		-1.3192	
hsa-miR-128	-2.6798	-2.7219	-0.8054	-0.9418	-3.6428
hsa-miR-129	-2.6873	-2.5092	-2.1439	-3.5065	-3.7659
hsa-miR-138-2	-2.9914	-3.7753	-3.5410		
hsa-miR-139	-2.8809	-3.4270	-1.4694		-2.5325
hsa-miR-218	-2.9017	-4.1373	-2.3114	-2.1683	
hsa-miR-29c	-1.4238	-2.0049		-2.5603	
hsa-miR-330	-2.3573	-2.7151		-1.9461	
hsa-miR-338	-1.2405	-2.7614	-3.3562		-3.0591
hsa-miR-383	-3.6230		-3.3105	-2.0549	-1.9873
hsa-miR-433	-2.3641	-2.3603	-2.3925		-2.8154
hsa-miR-485	-1.8770		-2.0218		-2.7502
hsa-miR-487b	-1.7351		-1.4938	-1.4251	
hsa-miR-491	-2.0305	-2.8435			-2.3616
hsa-miR-584	-1.3542	-1.8218		-1.9236	
hsa-miR-628		-1.9545	-1.4408	-1.7597	
hsa-miR-7	-3.2559	-5.3455	-1.5771		
hsa-miR-770	-1.7783	-3.2815	-2.8078	-2.6541	
hsa-miR-873	-0.8943	-2.9897	-3.3181		-5.2619
hsa-miR-885	-1.2288		-2.3533	-1.2555	

B

miRNA ID / ID Dataset	Alzheimer's Disease Datasets	
	GSE120584	GSE16759
Upregulated miRNAs		
hsa-miR-134	0.1569	2.1697
hsa-miR-185	0.2068	1.1829
hsa-miR-198	0.1407	3.4561
hsa-miR-659	0.1678	2.1595
hsa-miR-671	0.1869	2.9970
Downregulated miRNAs		
hsa-miR-29c	-0.2023	-1.4724
hsa-miR-494	-0.2013	-1.0508

Figure 1. (A) Differentially expressed miRNAs between GBM samples and normal brain tissues; (B) Differentially expressed miRNAs between samples of AD and normal controls. log2FC values relative to upregulated miRNAs were reported with a gradient of red boxes while a gradient of blue boxes were used for downregulated miRNAs. GBM, glioblastoma multiforme; AD, Alzheimer's disease.

to what it was observed for the GBM proteins clustered in the 'Molecular function' category (Fig. 5B). Finally, the analyzed proteins were part of the intracellular organelle, as well as the cell membrane, therefore playing a fundamental role in the regulation of cellular homeostasis (Fig. 5C).

Discussion

Despite tremendous advancements being made in the characterization of the clinicopathological features typical of tumors and neurodegenerative diseases, little is known about the association between the molecular mechanisms responsible for the development and progression of brain cancer and neurodegeneration (72-74). In detail, an indirect association in the incidence rates of GBM and AD has been widely reported, thus suggesting that this inverse association may be coupled with an inverse regulation of the same molecular mechanisms involved in the development of GBM and AD (29,30).

To shed light on the molecular mechanisms potentially responsible for the rise of these pathologies, and to demonstrate the existence of an inverse association between the molecular alterations in GBM and AD, the present study identified the miRNAs found altered in GBM and AD. In particular, in this study, we analyzed the potential involvement of these miRNAs in the onset of both diseases although oppositely expressed. For the first time, at least to the best of our knowledge, the existence of a strong inverse association was demonstrated in human samples between selected miRNA expression levels in GBM and AD through the comparison of altered miRNAs.

The choice of analyzing the expression levels of miRNAs in both pathologies depends on the numerous profiling data collected during the years for GBM and AD, and the increasing number of studies coupling the analysis of miRNAs in cancer and neurodegenerative disorders (75-78).

The independent analysis of GBM miRNA expression datasets revealed that 35 miRNAs were de-regulated in tumor samples compared to the normal controls. Almost all of these de-regulated miRNAs have been widely associated with the development and progression of GBM. In particular, several studies have already described the role of hsa-miR-21 and hsa-miR-155 overexpression in the promotion of the development of glioblastoma (79-81). Other studies have

miRNA ID	GBM Datasets					AD Datasets	
	Inverse miRNA expression patterns						
	GSE90604	GSE25632	GSE103229	GSE63319	GSE42657	GSE120584	GSE16759
hsa-miR-106a	1.5914	0.5187		1.0776	1.4142	-0.3209	
hsa-miR-20b	1.4360	0.8598		1.1470	1.8295		-1.8425
hsa-miR-424	1.1701	0.5340			1.1126		-3.6569
hsa-miR-1224	-1.1855	-3.7882			-3.5978	0.0815	
hsa-miR-129	-2.6873	-2.1439	-3.7659	-3.9761	-3.5065	0.1961	
hsa-miR-139	-2.4710	-3.1666	-2.5325	-4.0074		0.2056	
hsa-miR-330	-2.3573			-2.2716	-1.9311	0.2529	
hsa-miR-433	-0.5460	-2.3925	-2.8154	-2.3603		0.3400	
hsa-miR-485	-0.8953	-2.0218	-2.7502		-1.3304	0.3249	
hsa-miR-487b	-1.4497	-1.4938			-1.4251	0.3481	
hsa-miR-584	-1.3542			-1.8218	-1.9236	0.1494	
hsa-miR-885	-0.6140	-2.3533			-1.5306	0.3264	

miRNA ID	GBM Datasets					AD Datasets	
	Similar miRNA expression patterns						
	GSE90604	GSE25632	GSE103229	GSE63319	GSE42657	GSE120584	GSE16759
hsa-miR-29c	-1.4405			-2.0049	-2.5603	-0.2023	-1.4724

Figure 2. Comparison of the expression levels of miRNAs in GBM and AD datasets. In total, 12 out of 13 miRNAs exhibited inverse expression patterns in GBM and AD, while hsa-miR-29c was found to be downregulated in both GBM and AD. GBM, glioblastoma multiforme; AD, Alzheimer's disease.

miRNA ID	Glioma Pathway (hsa05214)		
	Inverse miRNA expression patterns		
	N° Genes	p-value	Genes
hsa-miR-106a	10	2.08E-28	PDGFRA, E2F1, NRAS, IGF1R, CCND1, PIK3R1, SOS1, AKT3, CDKN1A, **MAPK1**
hsa-miR-20b	9	3.13E-25	E2F1, PIK3R3, CCND1, PIK3R1, RB1, AKT3, CDKN1A, PTEN, MDM2
hsa-miR-424	19	2.97E-59	SOS2, CDK4, NRAS, **CALM3**, **CALM1**, PIK3CB, PIK3R2, IGF1R, CDK6, **CALM2**, CCND1, E2F3, AKT3, CDKN1A, MAP2K1, MTOR, **MAPK1**, GRB2, MDM2
hsa-miR-1224	2	3.84E-05	PIK3R3, CCND1
hsa-miR-129	4	2.18E-10	IGF1R, CDK6, CCND1, PTEN
hsa-miR-139	6	4.35E-16	CAMK2D, TGFA, RAF1, IGF1R, HRAS, PIK3CA
hsa-miR-330	3	1.07E-07	E2F1, IGF1R, **MAPK1**
hsa-miR-433	/	/	
hsa-miR-485	4	2.18E-10	CAMK2G, TP53, PIK3R1, CDKN1A
hsa-miR-487b	1	8.88E-03	MDM2
hsa-miR-584	1	8.88E-03	**CALM2**
hsa-miR-885	2	3.84E-05	IGF1R, PIK3R3

miRNA ID	Alzheimer's disease Pathway (hsa05010)		
	Inverse miRNA expression patterns		
	N° Genes	p-value	Genes
hsa-miR-106a	9	1.22E-17	CAPN2, EIF2AK3, PPP3R1, APP, ADAM17, FAS, SDHA, NDUFS7, **MAPK1**
hsa-miR-20b	4	4.14E-07	PPP3R1, APP, LRP1, ATP2A2
hsa-miR-424	23	5.27E-53	ATF6, GSK3B, **CALM3**, **CALM1**, APH1A, CDK5R1, COX5A, **CALM2**, NDUFC2, APP, NDUFA12, NDUFS8, ATP5G3, ATP5A1, ATP5G1, LRP1, UQCRH, ITPR1, ERN1, RYR3, APBB1, TNFRSF1A, **MAPK1**
hsa-miR-1224	2	1.59E-03	NDUFA10, ATP2A3
hsa-miR-129	2	1.59E-03	GSK3B, CAPN2
hsa-miR-139	2	1.59E-03	PPP3R1, PSEN1
hsa-miR-330	10	2.08E-28	ADAM10, APH1A, ADAM17, MME, NDUFB89, MAPK1, IDE, NDUFS7, **MAPK1**, BAD
hsa-miR-433	/	/	
hsa-miR-485	/	/	
hsa-miR-487b	/	/	
hsa-miR-584	4	4.14E-07	**CALM2**, BACE2, ATP2A2, UQCRB
hsa-miR-885	2	1.59E-03	PPP3CA, ATP5A1

miRNA ID	Glioma Pathway (hsa05214)		
	Similar miRNA expression patterns		
	N° Genes	p-value	Genes
hsa-miR-29c	11	1.22E-31	NRAS, CALM3, CDK6, TP53, PIK3R3, PIK3R1, SOS1, AKT3, CDKN1A, PTEN, MDM2

miRNA ID	Glioma Pathway (hsa05214)		
	Similar miRNA expression patterns		
	N° Genes	p-value	Genes
hsa-miR-29c	12	1.24E-24	GSK3B, CASP7, CALM3, BACE1, GAPDH, COX7A2L, ATP5G1, LPL, CASP8, TNFRSF1A, CYCS, ATP5G2

Figure 3. Diana-mirPath pathway prediction analysis showing the genes modulated by the selected miRNAs in glioma (hsa05214) and Alzheimer's disease (hsa05010) pathways. In red are reported the miRNAs upregulated, while in blue those downregulated. The genes shared in both glioma and Alzheimers' disease pathways are presented in bold.

demonstrated the therapeutic potential of the overexpression of several miRNAs normally downregulated in glioblastoma, such as hsa-miR-7, hsa-miR-93 and hsa-miR-139 (82-84). As regards the most downregulated miRNAs, hsa-miR-128 and hsa-miR-129, found in the GBM dataset analysis, some studies have highlighted their possible use in the context of novel therapeutic strategies aimed at inhibiting the molecular pathways involved in GBM aggressive phenotypes (85-87).

Concerning the analysis performed on the 2 AD miRNA datasets, the results revealed significant data for the downregulated miRNA, hsa-miR-29c. Several studies have demonstrated that the downregulation of this miRNA may play a potential role either as biomarker or as therapeutic agent in AD *in vitro* models and in patients (88-91). Of note, the downregulation of hsa-miR-29c has also been observed in GBM where it was observed that its induced overexpression led to the suppression of glioma (92).

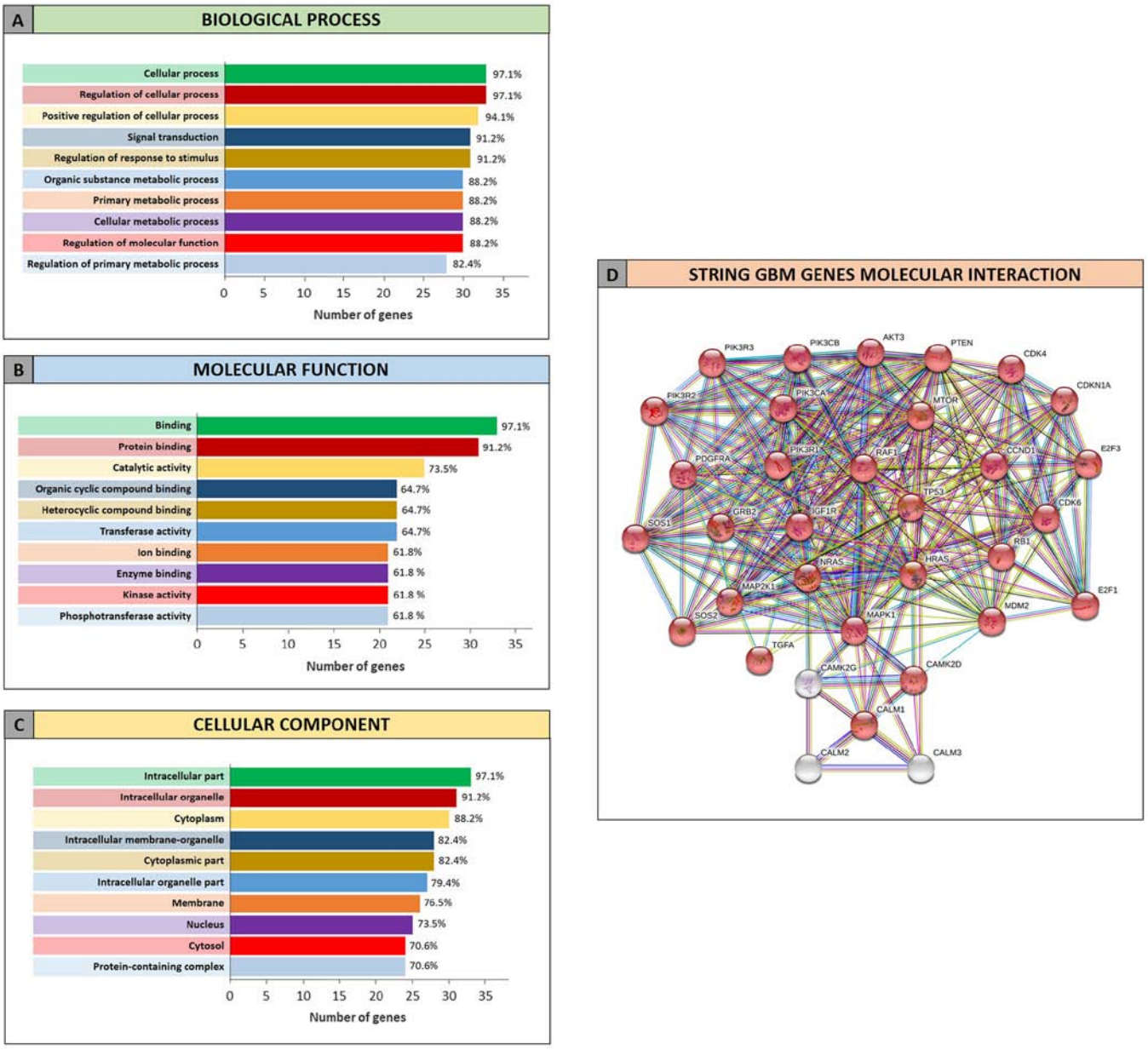

Figure 4. Gene Ontology enrichment analysis by STRING of miRNAs-targeted genes in GBM. (A) Genes clustered according to molecular function; (B) Genes clustered according to biological process; (C) Genes clustered according to cellular component; (D) Interaction network between genes targeted by the selected miRNAs in GBM. In red the genes involved in glioma pathway (hsa05214). GBM, glioblastoma multiforme.

However, the most robust and interesting data derived from the comparison between miRNAs de-regulated in both the GBM AD datasets, was that all miRNAs identified, with the exception of hsa-miR-29c, exhibited inverse patterns of expression in GBM compared with AD. This result is in accordance with the hypothesis of the existence of an inverse regulation of molecular pathways in GBM and AD, as postulated by Liu *et al* (30) and Sánchez-Valle *et al* (29). In line with this theory, miRNAs were found inversely regulated in the two pathologies by our analyses. Noteworthy, the inverse regulation of miRNAs in GBM and AD is coupled with the inverse regulation of targeted genes by selected miRNAs. Such inverse miRNAs and genes regulatory patterns may explain the inverse comorbidity existing between neurological disorders and cancers (6).

The preliminary data obtained comparing miRNA expression levels in GBM and AD were further confirmed by the pathway prediction and gene ontology enrichment analyses. In particular, the DIANA-mirPath analysis revealed that the 13 selected miRNAs were in common between GBM and AD and they were able to modulate several genes within the glioma pathway (hsa05214). In particular, the miRNAs were involved in the regulation of genes, such as MAPK1, IGF1R, and genes belonging to the PIK3 and RAS families, known to be involved in the development of GBM and other tumors (93-97).

Conversely, the same miRNAs were shown to also target fundamental genes involved in AD, such as APP, responsible for the β-amyloid plaque formation (98); GSK3B, hyperactivated in Alzheimer's neurons (99); NDUF family, responsible for mitochondrial alterations in AD (100); LRP1 able to regulate the metabolism of amyloid-β peptides thus maintaining brain homeostasis (101) and other genes.

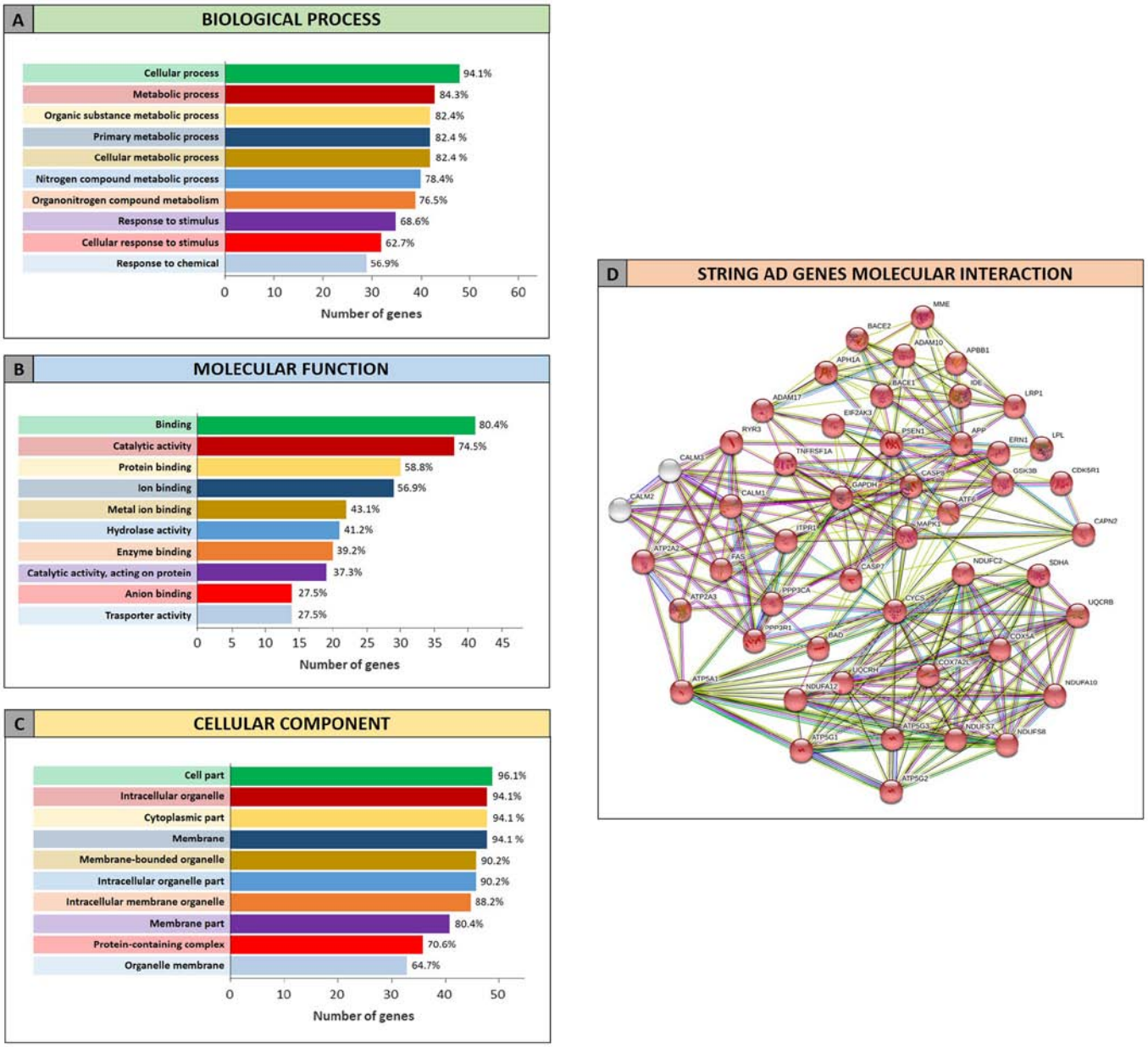

Figure 5. Gene Ontology enrichment analysis by STRING of miRNAs-targeted genes in AD. (A) Genes clustered according to molecular function; (B) Genes clustered according to biological process; (C) Genes clustered according to cellular component; (D) Interaction network between genes targeted by the selected miRNAs in AD. In red the genes involved in the AD pathway (hsa05010). AD, Alzheimer's disease.

Therefore, it is clear how miRNA de-regulation and other epigenetic mechanisms may lead to the modulation of these genes and, in turn, to the acquisition of a more aggressive tumor phenotype or neurodegenerative disorder susceptibility (102,103).

In addition, STRING analysis revealed that the selected miRNAs were able to modulate genes involved in both GBM and AD and those genes performed, in general, the same function and processes within the two pathologies. Therefore, differential expression levels of miRNAs in GBM and AD may be responsible for the onset of GBM rather than the AD, and vice versa.

Overall, the identification of altered miRNAs in both GBM and AD, as well as the definition of the inverse patterns of expression, may pave the way for new studies to better elucidate the involvement of these miRNAs in GBM and AD. In particular, further studies are required to examine the therapeutic potential of such identified miRNAs at the early stages of disease, since several treatments for AD are administered only when the pathology is at an advanced stage (104-106). On this matter, the results of the present study and the potential applications of this research methodology also to other brain diseases, may improve the diagnostic and therapeutic strategies mainly based on the analysis of low-specific biomarkers and on the use of low-sensitive and low-efficacy instrumental procedures (107-111).

In conclusion, in the present study, at least to the best of our knowledge, for the first time a set of de-regulated miRNAs in both GBM and AD was identified, demonstrating the existence of an inverse association between the expression levels of miRNAs in GBM and AD. The findings of the present study may pave the way for other functional *in vitro* and *in vivo*

studies to validate the diagnostic or prognostic significance of the identified miRNAs, as well as to depict their possible use as novel therapeutic approaches for GBM and AD.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request. The analyzed datasets are publicly available on the GEO DataSet database.

Authors' contributions

LF, DAS and ML conceived and designed the study. SC, LF, MP and GG performed all the analyses. GL, CDA and MSB provided all the information useful to describe the features of Glioblastoma Multiforme, while MP was involved in the analysis of AD datasets and gave information about neurodegenerative disorders and Alzheimer's disease. MP, MCP and SV were involved in the pathway prediction analysis and in the definition of the functional roles of selected miRNAs. LF, SC, MCP, MP and SV were involved in the interpretation of all data. GG, MCP and LF were involved in the preparation of the figures and tables. SV, LF and ML were involved in the preparation of the original draft of the manuscript, while MCP, SC, SV, GB, CDA and MP reviewed and edited the article. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All patient data were derived from publicly available datasets.

Patient consent for publication

Not applicable.

Competing interests

DAS is the Editor-in-Chief for the journal, but had no influence in the reviewing process, or any involvement in terms of adjudicating on the final decision, for this article. The other authors declare that there are not competing interests.

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