Chapter 4

Astrocytomas and miRNAs: Are They Useful?

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Additional information is available at the end of the chapter

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Abstract

Tumours in the central nervous system are a heterogeneous group of neoplasms originating in the neural ectoderm and other layers of the embryo. In the Children's Hospital of Mexico Federico Gómez, in accordance with what has been described in corresponding literature, these tumours occupy the third place, after leukaemia and lymphoma, in cancer cases. MiRNAs are non-codifying RNA molecules, of 18–24 nucleotides which regulate the expression of genes in a post-transcriptional level. Recently, the role of microRNAs (miRNAs) in the development of different types of cancer has been taken into consideration. In the case of astrocytomas, several target molecules of miRNAs have been determined, and their participation in the development of tumours has been proved since they are involved in differentiation, proliferation and apoptosis processes. MiRNAs are less susceptible to chemical modifications and degradement by ribonucleases by comparison with RNAm. The level of expression of miRNAs starting from bodily fluids represents the most promising advance for a non-invasive diagnosis and allows for their use as biomarkers to detect tumours in early stages and correlating them with clinical development.

Keywords: miRNA, CNS tumours, astrocytoma, brain, cancer, biogenesis

1. Introduction

Tumours in the central nervous system are a heterogeneous group of neoplasms originating in the neural ectoderm and other layers of the embryo. In the Children's Hospital of Mexico Federico Gómez, in accordance with what has been described in corresponding literature, these tumours occupy the third place, after leukaemia and lymphoma, in cases of cancer [1]. Fifty-five percent of patients are male. The predominant age was from older nurslings up to school-age children, with over 50% incidence. The tumours were 49% supratentorial and 51% infratentorial.

The most frequent ones were astrocytoma (32%), medulloblastoma (19%), craniopharyngioma (11%) and ependymoma (10%). In fifth place, there are germimoma (with 4%). Mixed glioma, primitive neuroectodermal tumours and ependymoblastomas made up 1–3% [1].

Tumour damage cause into the displacement of encephalic structures, oedema, tissue damage and the symptomatology are according to location, size and time of evolution in the tumour. Cephalea was the most frequent symptom in our hospital, followed by irritability, vomit and papilloedema. The growth of cephalic perimeter is of prognostic value in children less than 2 years of age [1].

Throughout the years, different classifications were postponed and applied for its study, based on histogenesis. Currently, the WHO has published its most recent classification [2] based on morphology and molecular changes. The reduction of costs and the increased ease of access to technology have made several medical centres approach this new era of molecular pathology research [2].

Particularly in the case of astrocytoma, the most frequent tumours in the central nervous system of children, there are several considerations. The diffuse astrocytoma group themselves

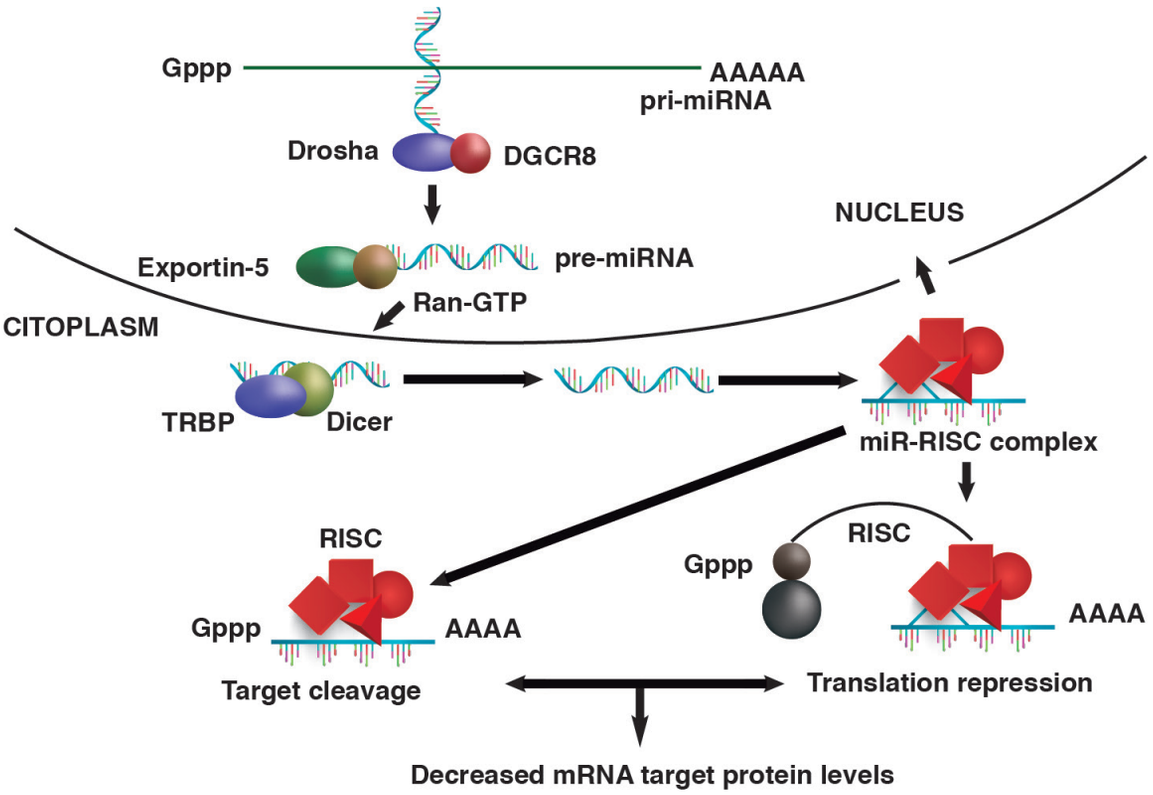

Figure 1. Biogenesis and functions of miRNA. MicroRNA is transcribed by an RNA polymerase II or III so as to generate a transcript of primary RNA within the nucleus. The stem-handle structure of pri-miRNA is recognised and cut by a complex microprocessor composed by Drosha and DGCR8 to produce a precursor microRNA of 60–70 nucleotides in length. Pre-miRNA is then exported to the cytoplasm through the nuclear pores, by means of exportin-5, and it is then processed in the cytoplasm by Dicer-TRBP. The RNA double-chain molecule is separated by a helicase of RNA. One of the strands of the miRNA/miRNA* duplex (the guiding strand or antisense strand) is incorporated, preferentially, in RISC, and it shall guide the miR-RISC complex towards the messenger RNA which shelters a complementary sequence to the miRNA. Once the RNAm is recognised, RISC may regulate the translation by inhibiting the starter or lengthening steps. In some cases, the miR-RISC complex may return to the nucleus [8].

today according to the expression of gene IDH1 or IDH2, which has enabled its correlation with prognosis [2].

Recently, the role of microRNAs (miRNAs) in the development of different types of cancer has been taken into consideration. MiRNAs are small RNA molecules that regulate the expression of genes in a post-transcriptional manner. This regulation is based upon a partial complementarity of microRNA with the target RNAm in such a way that it inhibits the synthesis of proteins (**Figure 1**). In the case of astrocytomas, several target molecules of miRNAs have been determined and their participation in the development of tumours has been proved since they are involved in differentiation, proliferation and apoptosis processes. It is also important to note that tumour cells in high-grade gliomas release microvesicles with miRNAs and proteins which can be detected in patients' serums. This makes miRNAs potential tumour markers. In the case of high-grade astrocytomas, the altered expression of several miRNAs such as miR-15b, miR-21, miR-34, miR-221, miR-10b, miR-124 and miR-181 has been reported, and their participation in the development of the tumour has been proven since they are involved in differentiation, proliferation and apoptosis processes [3–5]. In recent studies undertaken on the serum of patients with GBM, it has been observed that tumour cells release microvesicles, which contain miRNAs among which we can highlight miR-15b, miR-16, miR-21, miR-26a, miR-27a, miR-92, miR-93 and miR-320 [6].

Lages et al. [6] reported six microRNAs which clearly distinguish GBM from oligodendrogliomas. In GBMs, miR-21, -132, -134, -155, -210, and -409-5p were over-expressed. However, miR-128 was more expressed in oligodendrogliomas [7].

2. Importance of microRNAs

MiRNAs are non-codifying RNA molecules, of 18–24 nucleotides which regulate the expression of genes in a post-transcriptional level. They are found in a wide array of organisms, such as animals, plants and viruses, and in each type of cells [8, 9]. It is estimated that the genome of vertebrates codifies over 1000 different miRNAs, which regulate the expression of at least 30% of genes. The low necessary astringency for a functional interaction between miRNA/RNAm gives the capacity to miRNAs to regulate several messengers, besides region 3′UTR of target RNAm frequently harbouring several sites of recognition of microRNAs [10]. Close to 2588 mature sequences of miRNAs have been identified in the human genome [http://microRNA.sanger.ac.uk, version 21]. This number has rapidly increased in the last few years. Nevertheless, little is known about their specific goals and the biological functions that they undertake in the development of cancer and other illnesses [11].

3. Biogenesis

MiRNAs are initially transcribed as a long transcript known as primary miRNA (pri-miRNA) whose length goes between 3 and 4 kb, although some molecules may measure up to 10 kb. Pri-miRNAs are recognised in the nucleus by the complex composed by enzyme RNAse III

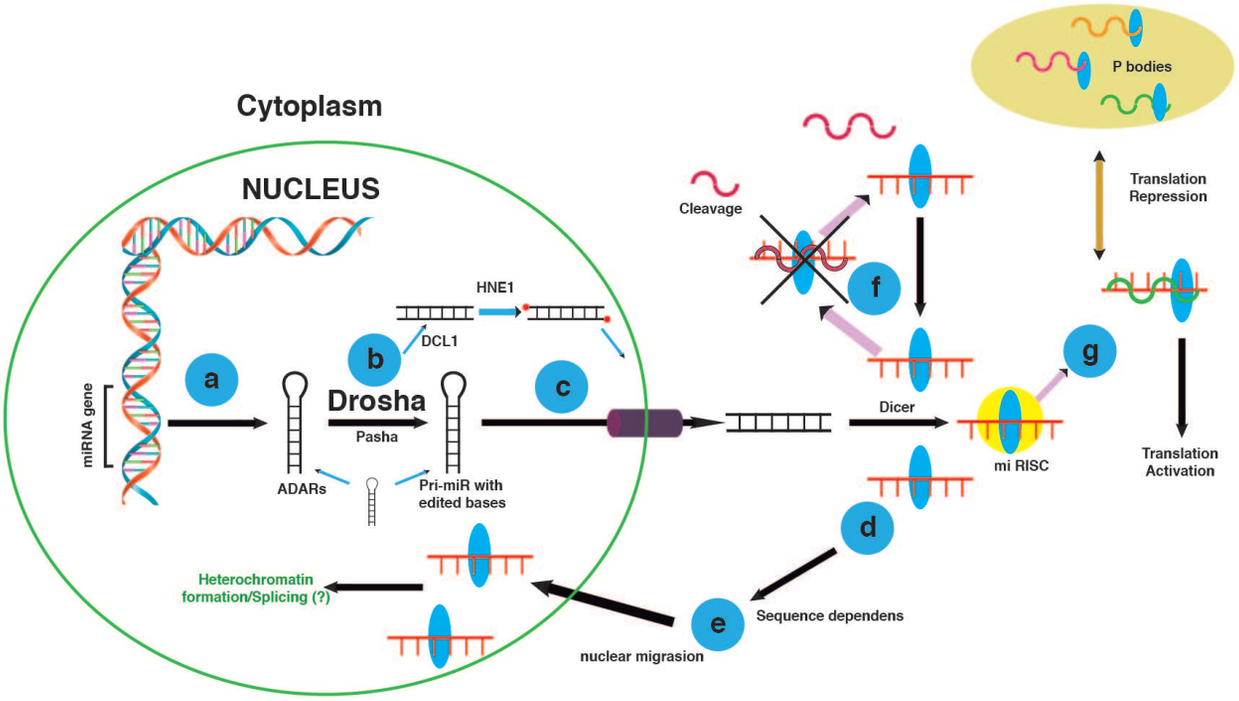

Figure 2. Biogenesis of the microRNA within the nucleus (a–c), maturing of the cytoplasm (d), formation of the microRNA-RISC complex which, depending on the sequence, shall return to the nucleus (e) or shall join its target RNAm to inhibit the translation (f) and finally the microRNA/RISC-RNAm complexes are stored in p-bodies where they are degraded or they return to the translation (g).

Drosha and DGCR8 (protein with a binding domain to double-strand RNA). This complex cuts the structure in a fork, becoming now a precursor miRNA (pre-miRNA) with a length of 60–80 nucleotides. The pre-miRNA is recognisable because of exportin 5 (nuclear exporting factor) and the nuclear protein Ran-GTP. Both transport pre-miRNA towards the cytoplasm. The Dicer and TRBP enzymes (proteins with binding domain to RNA) undertake a second cut in the base of the stem-handle and they generate an RNA molecule, double strand, of 18–24 nucleotides in size [12]. A great protein complex known as silencing complex induced by RNA (RISC) is associated with duplex RNA and separates both chains. RISC is a tetrameric complex made up of Dicer, TRBP (protein with binding domain to RNA), PACT (Activating Protein) and Ago2 (Protein of the Argonaut family). Ago2 identifies the target RNAm based on the complementarity with the associated single-strand microRNA. Recognised sequences of target RNAm are located mainly in region 3, non-translated (3′UTR). Generally speaking, only one strand is incorporated within RISC and the other one is downgraded. This miRNA guides RISC towards the target messenger inhibiting its translation (**Figure 2**) [13, 14].

4. MiR/RISC-RNAm complex

The recognition of the target RNAm takes place because of the complementarity of the sequence known as ‘seed’ (nucleotides 2–8) located in the 5′ extreme of the microRNA with the sequence of the target RNAm. Recognised sequences of target RNAm are in region 3′,

non-translated (3′UTR) (60%), in codifying sequences (25%), in introns, in non-codifying RNA sequences and in 5′UTR. The degree of complementarity between microRNA and RNAm determines the silencing mechanism. When complementarity is 100%, targeted RNAm is downgraded, which mostly happens with plants. In animals, complementarity is 100% in the seed region, but not throughout the microRNA in such a way that a mechanism of inhibition of the target messenger takes place.

The effector complex headed by the Argonaut protein probably interacts with translational systems to inhibit the synthesis of proteins at the beginning or in the elongation step, depending, probably, on the nature of the miRNA and the target transcript [13, 14]. Most microRNAs described in the human body exert their inhibiting effect in the cytoplasm; nevertheless, there exist some microRNAs as miR-29b which has a terminal sequence of hexanucleotides which allows it to return to the nucleus where it possibly undertakes its functions.

The complexes formed by the microRNA/RISC-RNAm do not remain indefinitely in the cytoplasm, but they are rather transported towards structures of cytoplasmic processing, called P-bodies, where the downgrading of RNAm may take place due to deadenylation and decapping, or it is also stored and then separated from the repression complex and the P-body and returns to the translational machinery (**Figure 3**).

MicroRNAs participate in fundamental cell processes such as determining the cell lineage, apoptosis, proliferation, migration and regulation of the cell cycle, in which the translation of specific genes is highly precise and coordinated. MicroRNAs make up complex regulatory networks with its target genes, representing common mechanisms that have evolved

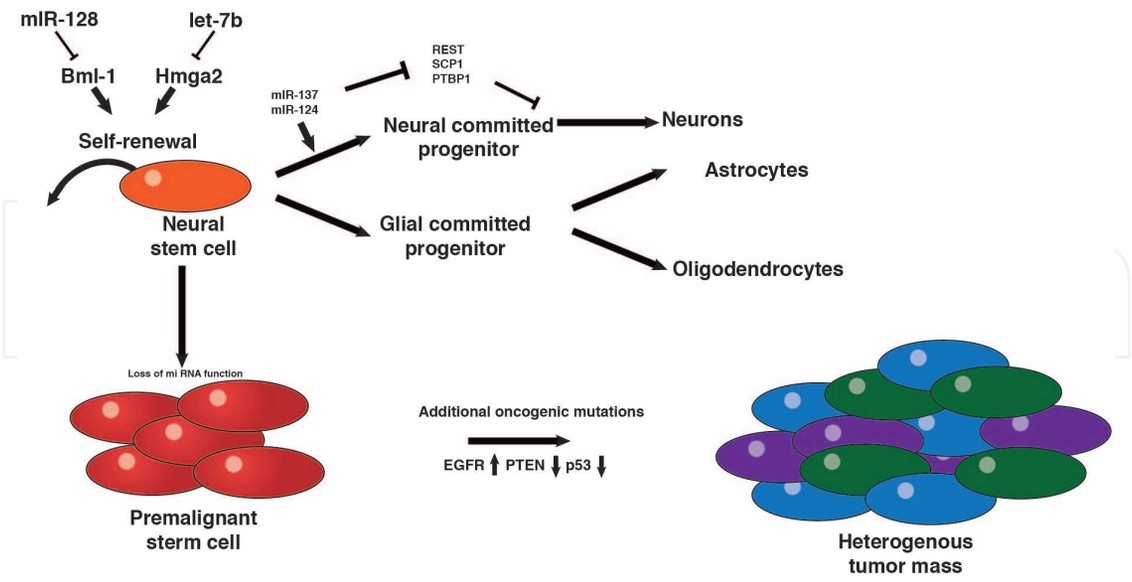

Figure 3. Model which shows the effects of deregulation of miRNAs in the development of the glioma. Under normal physiological conditions, the expression of miRNAs is important for the induction of differentiation in the SNC and abrogation of the self-renewal of the stem cell. The loss of expression of miRNAs results in the creation of pre-malignant stem cells, which are hyperproliferative and non-differentiated, which may progress into a glioma of low to high grade. Additional oncogenic mutations may facilitate the malignant phenotype.

in mammals strengthening genetic regulation. At the same time, microRNAs are regulated by oncogenes, tumour-repressing genes, epigenetic mechanisms, genetic abnormalities and defects in the miRNA biogenesis machinery [15]. Each one of these mechanisms may contribute by themselves or, more likely, together, to alter the expression of miRNAs in cancer [15, 16].

5. Patterns of expression for miRNAs in the brain

The central nervous system of mammals is controlled importantly by genetic regulation mechanisms. MicroRNAs contribute to this regulation; approximately 70% of identified miRNAs until now are expressed in the brain and some of them are specific to the brain [17]. In recent studies, the pattern of expression for microRNAs was determined and it was shown that they regulate both development and functionality of the nervous system [9, 18].

A wide variety of microRNAs are in neuronal subtypes with the highest concentration in the brain cortex and cerebellum [19, 20]. In the central nervous system, there are a large number of genes which originate miRNAs and their expression is different depending on the anatomical region. Specific microRNAs for the brain are miR-9, mir-124, miR-125, miR-128 and miR-129 [21–25]. MiR-124 and miR-128 are expressed mainly in neurons, whereas miR-23, miR-26 and miR-29 can be found enriched in astrocytes [10, 26, 27]. In the same way, the expression profile of miRNAs in the development and differentiation of the nervous system in mammals is fundamental, since changes have been documented in their expression when embryo stem cells develop neurogenesis and gliogenesis, which suggests that they may have an important role in differentiation or determination of the cell lineage [9, 14, 22, 28, 29].

6. MicroRNAs and their relationship with cancer

Calin et al. were the first ones to find evidence regarding the relation between miRNAs and cancer, demonstrating that miR-15 and miR-16 are located in a mutated region, in over half of chronic lymphocytic leukaemias in B-cells [30]. Several following studies have demonstrated that the expression profiles of several miRNAs are altered in different types of tumours such as glioblastoma, pituitary adenoma, prostate cancer, breast carcinoma, hepatocellular carcinoma, lung carcinoma, colorectal carcinoma, ovarian carcinoma, thyroid and cervical carcinoma, lymphoma and chronic lymphocytic leukaemia [31–35]. For this reason, some of them are considered tumour-suppressive genes or oncogenes [36–38]. Genetic events guiding the development of tumours in the brain are yet unknown; nevertheless, there is evidence which suggests that gliomas may surge starting from a subpopulation of cells within the tumoural mass; these cells have been called ‘stem tumour cells’, which maintain their ability for renewal and multi-potentiality. MiRNAs are important regulators of the process of differentiation and proliferation of stem cells (**Figure 4**) [39–41].

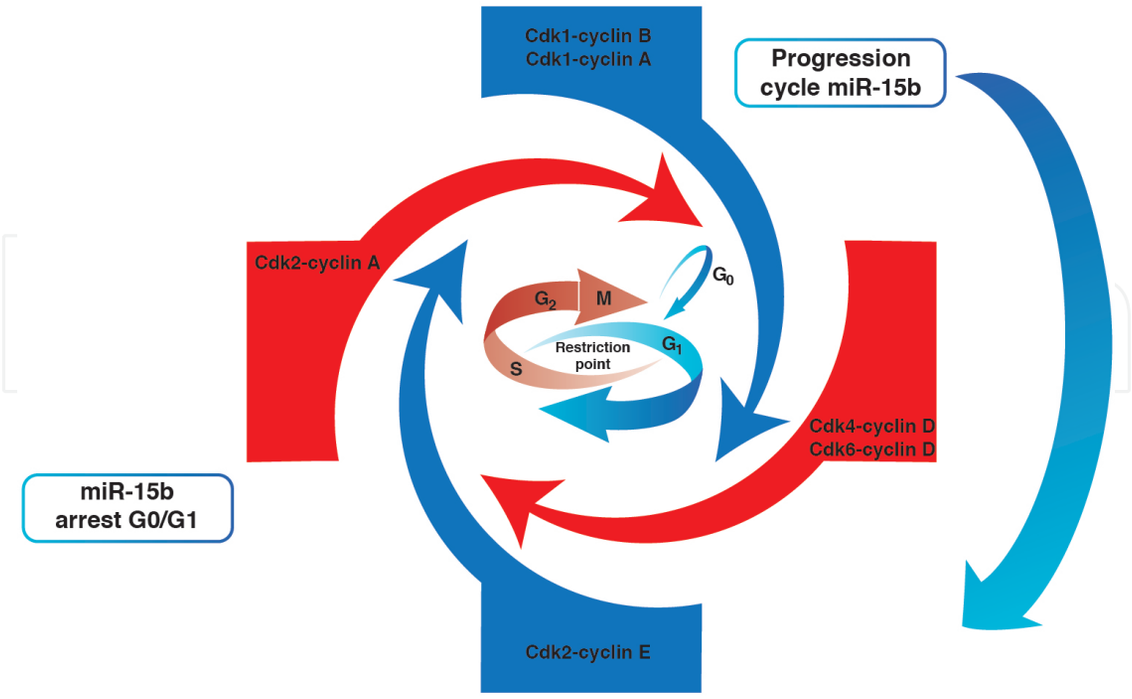

Figure 4. Mir-15b regulates the progression of the cell cycle because it has cyclin E as a target. The over-expression of miR-15b causes an arrest of the cell cycle in the G0/G1 phase, whereas the low expression causes a reduction in the population of cells in G0/G1 and an increase in phase S [51].

7. Expression profile for miRNAs in astrocytomas

Different expression patterns in miRNAs have been described in low- and high-grade astrocytomas including pilocytic, diffuse, anaplastic astrocytomas, and multi-form glioblastoma in adults. In these tumours, miRNAs participate in the cell proliferation, invasion, angiogenesis and differentiation [42, 43]. The first reports are very recent and started with the identification of miRNAs in the GBM in 2005. In this type of tumour, an overexpression of miR-221 was described and proposed as a possible specific marker, whereas miR-128, miR-181a, mir181b and miR-181c were found to be low expression, which probably reflects a loss of expression associated to the lack of differentiation in tumour cells [38]. In that same year, an over-expression of miR-21 in GBM and cell lines was described, comparing it with normal tissue. These effects were related with a reduction of apoptosis and malignant phenotype. On the contrary, the low expression of miR-21 promoted the activation of caspases and apoptosis [44]. Afterwards, in another study, miR-124 and miR-137 were identified, related with the neuronal differentiation in mouse stem cells, derived from a mouse oligodendroglioma and derived of human GBM. Besides, in a cell line of GBM, arrest in the cell cycle after transfecting miR-124 and miR-137 could be observed, which suggests that miR-124 and miR-137 may be target molecules for therapeutic treatments of this illness [44]. These studies suggest that miRNAs participate in multiple biological processes which are characteristic of GBM such as cell differentiation, proliferation, invasion, apoptosis and angiogenesis. Given that miRNAs may promote or limit the development of the tumour, they may be considered as having oncogenic potential or tumour-suppressive activities. MiRNAs

analysed in this study and which are considered in the study as having oncogenic potential are miR-15b, miR-21 and miR-221 and the tumour suppressors are miR-124, miR-128, miR-137 and miR-221. Next, each one of them is described [5, 23, 45–48].

8. MicroRNAs with oncogenic potential: antiapoptotic and proliferative functions

8.1. MiR-9

The gene that codifies miR-9 is located in the genome of three different regions: miR-9-1 is located in the 1q22 chromosome, miR-9-2 in 5q14.3 and miR-9-3 in 15q26.1. This miRNA is expressed almost exclusively in the brain and it is a neurogenetic mediator. In the fetal brain, it is highly expressed, compared with that of an adult [49]. Nass et al. studied the expression of several miRNAs in primary brain tumours and metastatic brain tumours through micro-arrangements and qRT-PCR, and they observed that miR-9/9* were mainly overexpressed in primary brain tumours, by comparison with metastatic brain tumours, and they concluded that it is possible to distinguish between both types of tumours with a high degree of reliability [50]. Up until now, it has only been described as one of its targets for the REST transcription factor.

8.2. MiR15b

Located in chromosome 3q25.33, Xia et al. identified a panel of miRNAs expressed differentially in glioma tissue. One of the significantly deregulated miRNAs was miR-15b. Afterwards, they identified their potential targets being CCNE1 (protein related with the transition of the cell cycle of G1/s) as one of them. The levels of expression of RNAm of CCNE1 in the cell lines after transfection with exogenous miR-15b were analysed, as the anti-senses of miR-15b, and they observed that the levels remained without changes. Nevertheless, protein levels of CCNE1 were significantly reduced after the transfection with exogenous miR-15b and they were increased after transfecting the antisense of miR-15b. These results suggest that CCNE1 is a potential target. The overexpression of this miRNA causes arrest in the cell cycle in its G0/G1 phase, whereas its inhibition results in a reduction of the cell population in G0/G1 and therefore also represents an increase in phase S (**Figure 5**) [40].

8.3. MiR-21

The gene which codifies for the miR-21 is located in chromosome 17q23.1. The overexpression of this miRNA was described for the first time in the GBM and afterwards in other types of solid tumours [31, 44]. Chan et al. studied the expression of miR-21 in patients with GBM and in cell lines of gliomas and observed that, in tissues, the expression of miR-21 was increased five to 100 times in comparison with non-neoplastic brain tissues. There are several important targets which contribute to its anti-apoptotic and proliferative actions, such as some molecules involved in the suppressor tumour routes for p53, TGF-β (β-transforming growth factor) and a mitochondrial apoptotic route [52–54]. In a recent study, developed with 124 samples of astrocytomas of high and low grade, it was found that miR-21 is more sensible to predict the clinical development of

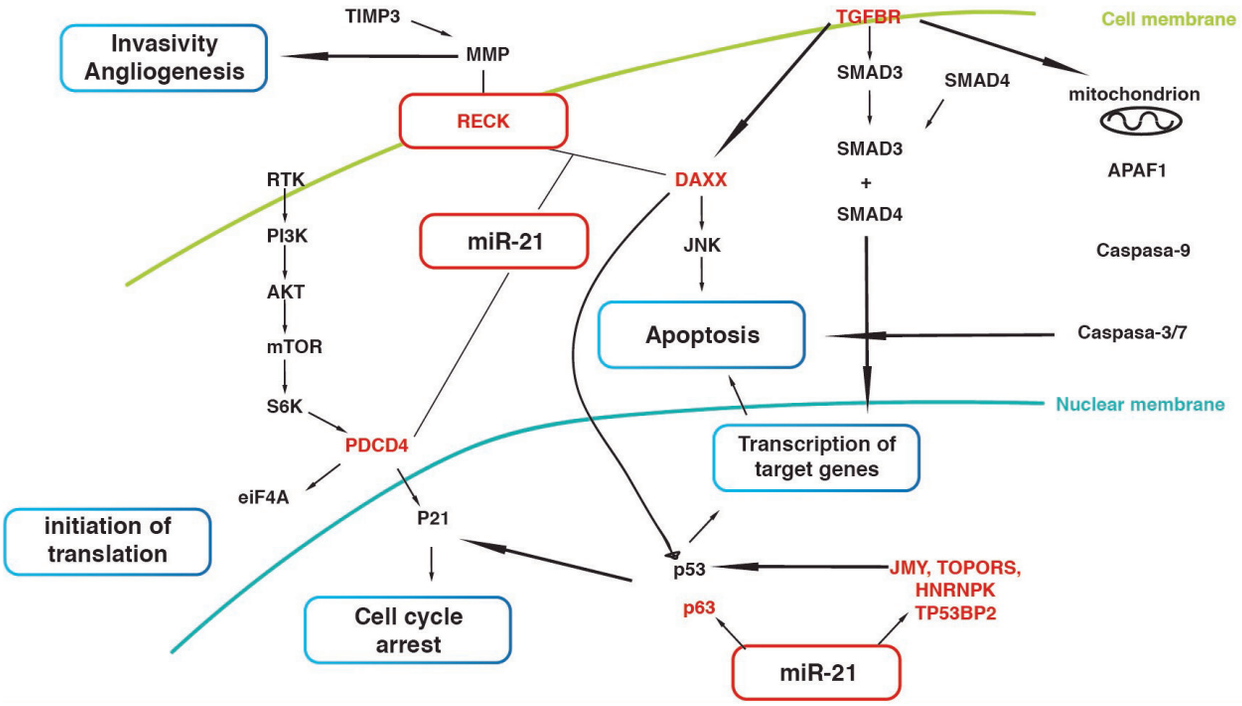

Figure 5. Signalization routes influenced by miR-21 in glioblastoma cells. MiR-21 regulates apoptosis, cell cycle and translation [52].

high-grade astrocytomas, because they observed a greater expression in high-grade tumours and a lower survival rate compared with low-grade astrocytomas [43]. It is evident that the over-expression of miR-21 in astrocytomas results in the activation of multiple oncogenic routes [57]. Many other studies have confirmed the over-expression of this miR in the four grades of astrocytomas and in other tumours of the SNC as oligoastrocytoma, oligodendroglioma and medulloblastomas, having a greater expression in the multi-shaped glioblastoma (**Figure 6**) [26, 38, 58].

8.4. MiR-221/222

MiR-221/222 are located in chromosome Xp11.3 and they are over-expressed in astrocytomas, their expression is co-regulated and they have the same specificity of targets because the region considered as "origin" or "seed" region is the same in both cases [7]. Ciafrè et al., through microarrangements of expression and Northern blot, analysed nine samples of patients with GBM and 10 cell strands of glioma and identified miR-221 as one of the miRNAs with greatest overexpression in comparison with values obtained in normal brains and samples of healthy tissue that were close to the tumour [38]. Gillies et al. 2007 described $p27^{kip1}$ as a direct target of miR-221/miR-222. $P27^{kip1}$ is a protein that regulates the cell cycle, its function is inhibiting the cyclin-depending kinase (CDK) in such a manner that there is an arrest in the cell cycle in the phase G1, avoiding cell proliferation [59] (**Figure 5**). Medina et al. studied the participation of several microRNAs in the regulation of the cell cycle and observed that the expression of miR-221 and miR-222 was increased in human quiescent cells which are stimulated for proliferation. They predicted and proved two targets: p27 and p57; both suppress the cell growth because they inhibit cyclin-dependent kinases. The over-expression of these miRs is closely linked to the control of the cell cycle, which assures

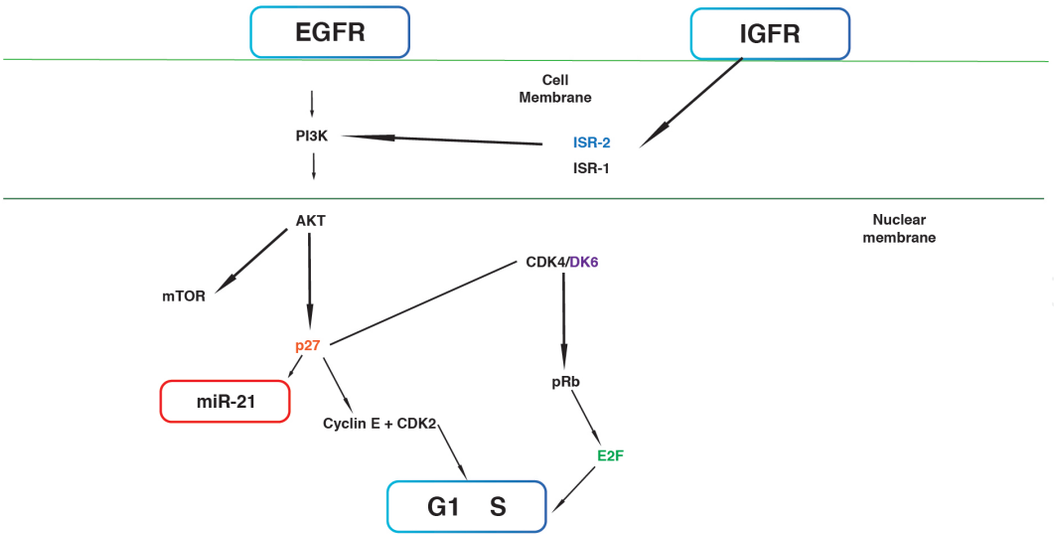

Figure 6. The miR-221 oncogene promotes the progression of the cell cycle because it inhibits the translation of the tumour suppressor $p27^{kip1}$, whose reduction causes the expression of CDK and, with it, the progress of the cell cycle [52].

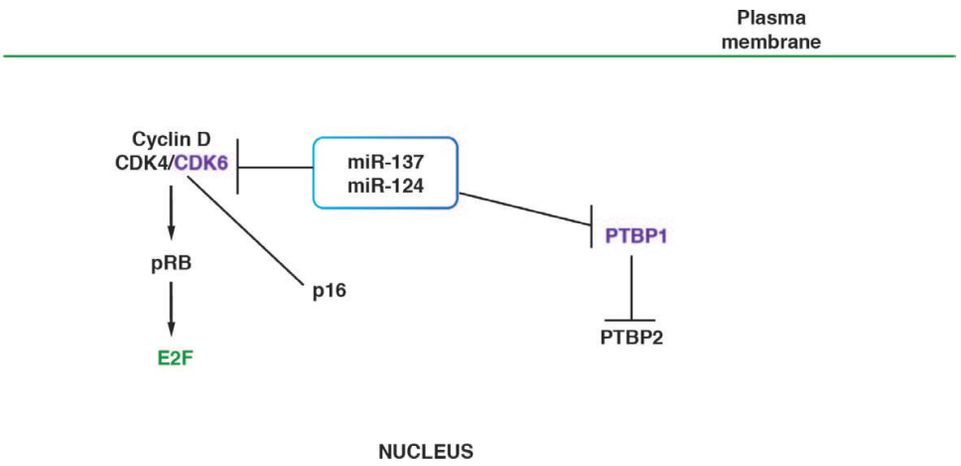

Figure 7. MiR-124 and miR-137 has CDK6 as target, CDK6 which is a regulator of the cell cycle and differentiation. PTBP1 is also one of its targets and is related with alternative 'splicing'.

the survival of the cell by a coordinated competence between the entrance in phase S and signalization routes of the growth factor that stimulates the cell proliferation [55]. The high expression of miR-221 in high-grade astrocytomas and cell strands, and they strongly imply that it is a candidate to becoming a specific tumour marker (**Figure 7**) [59].

9. Tumour-suppressing microRNA: neural differentiation and proliferation

9.1. MiR-124

There are three genes that codify for miR-124 and are located in different regions; thus, we have miR-124-1 located in chromosome 8p23.1, miR-124-2 located in 8q12.3 and miR-124-3 in

20q13.33. It is the most profuse brain-specific miRNA; during neural differentiation, it expresses itself mainly in neurons [49]. It is considered a tumour suppressor weakly expressed in anaplastic astrocytomas and GBM, in relation with the non-neoplastic brain tissue. In this regard, Silber et al. studied the expression of several miRNAs during the differentiation of adult neural stem cells, and it was observed that miR-124 increased its expression eight times, instead of what happens in high-grade tumours, where their expression is less. In this same study, they also determined that miR-124 may induce differentiation and inhibit the proliferation of glioblastoma stem cells when inhibiting CDK6 (cyclin 6, dependent on kinases) which, as a goal, promotes the progress of the cell cycle (**Figure 8**) [45, 54].

9.2. MiR-128

MiR-128-1 is located in chromosome 2q21.3 and miR-128-2 in 3p22.3. It is an miRNA specific to the brain, where it finds itself enriched. On the other hand, in gliomas and glioma cell strands, its expression is lowered [25, 38, 42]. Zhang et al. studied the expression of miR-128 in astrocytomas GII, GIII and GIV and in cell strands, and they observed that it lowers itself progressively as the grade of the tumour increases. Its tumour-suppressing characteristics were evidenced when transfecting miR-128 in glioma cell strands, observing an inhibition in cell proliferation [25]. Godlewski et al. proved the low expression of miR-128 in gliomas and in cell strands and focused in finding a target that was related with cell differentiation and self-renewal. MiR-128 makes up for an important biological target against the 'tumour stem cells' which are characteristic and part of the origin of the glioma (**Figure 9**) [42].

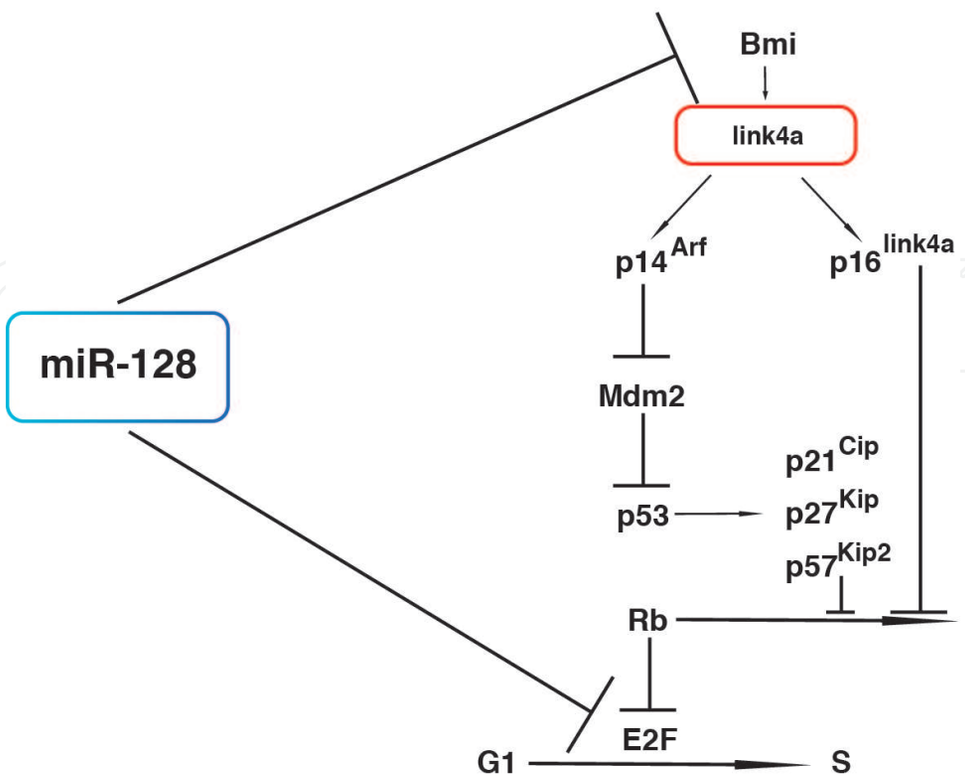

Figure 8. MiR-128 has Bmi and E2F as its main targets.

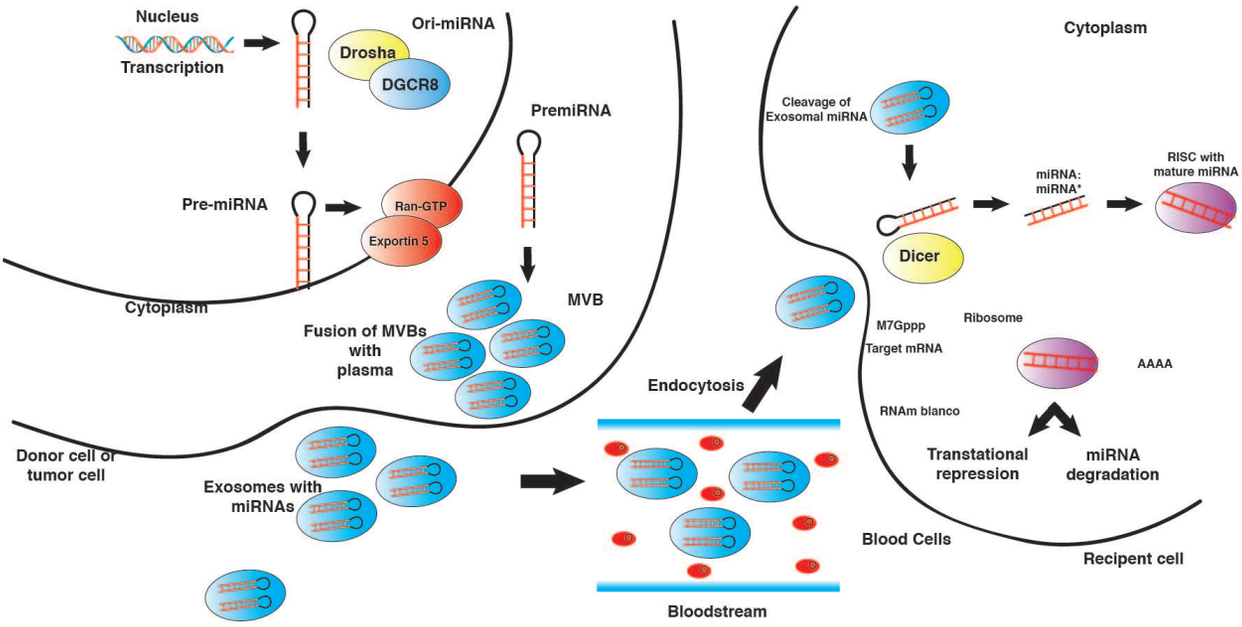

Figure 9. Possible transport route of miRNAs in serum, and their final destination to receptor tissue cells.

9.3. MiR-137

Located in the chromosome 1p21.3, Silber et al. studied the expression of several miRNAs during the differentiation of adult neural stem cells and observed an increase of miR-137 24-fold. This miRNA is considered a strong anti-proliferation factor and a cell pro-differentiator, with tumour-suppressing activity in gliomas, and may be of therapeutic relevance [42]. In high-grade astrocytomas, the expression of miR-137 is lowered. One of its validated targets through the reporting system of luciferase is CDK6, which regulates the progress of the cell cycle and differentiation, suggesting that miR-137 mediates the inhibition of CDK6, which can, in part, cause proliferation and differentiation of CBM cells (**Figure 8**) [54].

9.4. MiR-181

The miR-181 family is made up of miR-181a located in 9q33.3, miR-181b in 1q32.1 and miR-181c located in 19p13.13; miR-181a and miR-181b are enriched in a normal brain. Ciafre et al. studied the expression profile in patients with glioblastoma, finding a low expression of miR-181a, miR-181b and miR-181c in 20–30% of cases. In cell strands, a low expression was also observed, being miR-181a the one with the lowest expression, followed by miR-181b. In this case, low expression was correlated with the lack of differentiation of tumour cells [38]. In the same manner, Shi et al. studied a small series of gliomas in grades II, III and IV and observed a low expression of miR-181a and miR-181b associated with the grade of tumour. They also transfected glioma cell strands with both miRs and they observed an inhibition of the growth, induction of apoptosis and inhibition of the invasion. These effects were more evident with miR-181b [55, 56]. Conti et al. studied the expression of miR-181 in different grades of astrocytoma from a diffuse astrocytoma, grade II up to GBM GIV and observed the low regulation of mir-181b in all grades; nevertheless, the expression levels of miR-181a and miR-181c were similar to those on a normal brain [24]. Zhi et al. studied a total of 124 astrocytomas ranging from GI to GIV and they found low levels of miR181b which were associated with low survival. The authors also mention that miR-181b

is the most sensible way to predict the clinical diagnosis for patients with low-degree astrocytomas. These results suggest that miR-181 may maintain the state of differentiation in normal brain cells for which their diminution would induce the loss of differentiation in tumour cells. The identification of target may provide information regarding the cell differentiation (**Table 1**).

The findings that are registered in the study up to now represent starting studies; nevertheless, it has been established that the deregulated expression of miRNAs participates in the tumourigenesis in several types of tumours such as GBM. Data are scarce regarding the differential expression of miRNAs in low- and high-grade astrocytomas in children. In children, low-degree astrocytomas are the most common; nevertheless, high-grade astrocytomas take place frequently and in advanced clinical studies. In paediatric population, the profile of expression of miRNAs in low- (GI and GII) and high-grade astrocytomas (GIII and GIV) is unknown. With methods of cell and molecular biology, it is possible to generate information regarding the biological behaviour of these molecules and to establish molecular markers which may be used to identify and differentiate the different grades of astrocytomas that have malignity characteristics, despite being low grade. The goal of this work is to determine the

miRNA	**Normal brain**	**Type of glioma and expression**	**Biological function** ***Oncogene/tumour suppressant***	**Target RNAm** **Experimentally validated**	**Number of possible targets**
MiR-9	Abundant	High GIV	Differentiation *Oncogene*	REST	683
MiR-21	Basal	High G II, III, IV	Proliferation and anti-apoptosis *Oncogene*	p63, JMY, TOPORS, TP53BP2, TGFβR2/3, DAXX, HNRPK, PDCD4, RECK, TIMP3, LRRFIP1	210
miR-221	Basal	High G II, III, IV	Proliferation: cell cycle *Oncogene*	CDKN1B/p27 CDKN1C/p57, BIRC1	307
miR-15b	Basal	High (cell strand glioma U118)	Regulates the progression of the cell cycle (arrest in G0/G1) *Oncogene*	CCNE (codifies cyclinE1)	968
MiR-124	Abundant, specific	Low G III, IV	Differentiation, proliferation: cell cycle *Suppressor tumour*	PTBP1 (neural differentiation), CDK6	1299
miR-128	Abundant, specific	Low G II, III, IV	Proliferation: cell cycle *Tumour suppressor*	E2F3a, BMI1	785
MiR-137	Abundant	Degrees III and IV, low	Induces differentiation, inhibits proliferation *Tumour suppressor*	CDK6	468
miR-181a	Abundant	G II, III, IV, low	Induces apoptosis, inhibits invasion and growth *Tumour suppressor*	Not reported	892

Table 1. Expression of microRNAs in normal brain and in astrocytomas, their functions and validated targets.

profile of expression of miRNAs present in low-grade (G I, II) astrocytomas and in high-grade astrocytomas (G III, IV) in paediatric population.

10. Expression of microRNAs in serum

One of the goals within cancer study is to develop non-invasive tests for the diagnosis and follow-up of patients; because of this, there is a great interest in the detection of nucleic acids that are circulating in serum and plasma. Serum and plasma contain a great number of stable miRNAs, despite the high content of ribonucleases in the plasma. This stability may be given by finding itself within the exosomes (organelles derived from endosomes), by chemical modifications or by associating with protein complexes such as RISC [60, 61]. Lawrie et al. [4] reported their first study regarding miRNAs, associated with tumours, in lymphoma patients' serums, and they found that the levels of miR-155, miR-210 and miR-21 were higher than those found in control serums of healthy patients. In this study, they related the high expression of miR-21 with a better prognosis. These results were consistent with previous results in biopsy material from lymphoma patients, in which high levels of miR-21 were associated with a better prognosis [4]. Chen et al. detected and sequenced 100 miRNAs in healthy patients' serums and in patients with lung and colorectal cancers, reporting specific expression patterns of tumour type. In this same study, they distinguished the miRNAs in the serums of other species of small nucleotides such as tRNA or downgraded RNA fragments, concluding that miRNAs are the main fraction present in serum [62, 63]. One of the first undertaken studies in astrocytoma patient serums was the one by Skog et al. in which they report that tumour cells on glioblastomas release microvesicles that contain microRNA, RNAm and angiogenic proteins [64]. These results indicate that patients with cancer present elevated levels of exosomes in plasma, derived from the tumour, in comparison with controls. Although normal cells may contribute to the population of exosomes in the peripheral circulation, the main source of circulating exosomes in cancer patients is originated in the tumour. Nevertheless, little is known about the mechanism by which miRNAs are generated in plasma and the biological impact of these molecules in distant sites of the body [61]. The discovery of miRNAs in serum opens the possibility of using them as biomarkers in different illnesses.

11. Regulator mechanisms of miRNAs

The regulation of miRNAs in cancer is undertaken by multiple mechanisms such as transcriptional regulation, epigenetic alterations, mutations, abnormalities, in the number of copies in DNA and defects in the biogenesis machinery for miRNAs. Each one of these mechanisms may contribute by themselves, or more probably to alter the expression of miRNAs in cancer [11, 15, 65]. Up next, each one of these regulation mechanisms is detailed.

The *transcriptional regulation* contributes to the alteration of expression patterns in miRNAs, an important example is that of miR-34b, a tumour suppressor which is regulated by the transcriptional factor p53. The inactivation of p53 in gliomas reduces the expression of miR-34, which makes it inhibit the cell proliferation, the progression of the cell cycle of G1/s, cell survival,

migration and cell invasion [66], and correction. Another example is miR-451; in this case, it is known that there are two transcription factors, SMAD3 and SMAD4, separated by 157 pb and whose binding sequence is in 1135 pb upstream from the miR-451 sequence. Both factors increase the transcription of miR-451 and induce the inhibition of growth and proliferation [67].

Epigenetic mechanisms may regulate up to a certain degree, the imbalance of miRNAs in tumour cells [68]. The methylation of DNA and modification of histones play a predominant role in the remodelling of chromatin and the general regulation of expression of genes that codify proteins. The hyper-methylation of CpG islands associated with specific miRNAs has been proposed as one of the mechanisms by which a low expression of miRNAs in tumour cells has been observed. The epigenetic silencing of miRNAs that act as tumour suppressors is emerging as an important alteration in cancers. Lujambio et al. studied the expression profile for several miRNAs in cells derived from a metastatic ganglion, and afterwards, the cells were treated with a de-methylating agent, observing that there was some re-expression of some miRNAs such as miR-148a, miR-34b/c and miR-9 [68]. The regulation of miR-124 is given, partly, due to epigenetic mechanisms, which was observed in a cell strand for colon cancer. No expression of miR-124 was observed here, but when cells were treated with a de-methylating agent, their expression was restored and, at the same time, correlated with the inhibition of one of its targets, CDK6. This result is due to miR-124 being located within a great CpG island, which, in a normal colon tissue, would be hypo-methylated, but in colon, tumour finds itself hyper-methylated [69]. In the same manner, the epigenetic silencing of miR-124 was evidenced when treating glioma cell strands with 5-aza-2′-deoxicitidine (a methylation inhibitor) and TSA (histone deacetylase inhibitors), increasing the expression of miR-124 [45]. In gliomas, miR-137 is partially regulated by epigenetic mechanisms, and its expression was increased 12-fold when astrocytoma cell strands were treated with de-methylating agents. This suggests that epigenetic modifications for regulating sequences in CpG islands may contribute to silencing miR-137 in GBM [45] (**Figure 10**).

Somatic mutations and/or in the germinal line, identified in miRNAs, are scarce. Some of the most recent findings have taken place in chronic lymphocytic leukaemia (CLL) [30]. In this illness, 42 genes which codify microRNAs were sequenced and five microRNAs with mutations were found. In the case of solid tumours, 15 miRNAs were evaluated in 91 epithelial-origin tumour cell strands and mutations were found in one case, a variation in the sequence of the precursor miRNA, and 15 variations in the sequence of primary miRNAs [15]. These mutations may be found in pri-, pre- and mature sequences of miRNAs [16].

The *abnormality in the number of DNA copies* is one of the mechanisms which modify the expression and functioning of genes. It is calculated that close to 50% of genes that codify human miRNAs and are registered are located in fragile areas, in regions with minimal loss of heterozygosity (LOH), minimal amplification regions and breaking regions. In chronic lymphocytic leukaemia, region 13q14 is deleted in over 50% of cases, and in this place, there is miR-16-1 and miR-15a. These two miRNAs have Bcl-2 as a target and work as tumour suppressors in this illness. The deletion of these miRNAs has also been identified in pituitary adenomas, ovary adenomas and breast cancer. In patients with lymphoma, the amplification of C13orf25 located in 13q31-32 has been described; in it, seven polycystronic miRNAs have been located. This group of miRNAs work as oncogenes, altering the balance between apoptosis and proliferation through the proto-oncogene c-Myc [15].

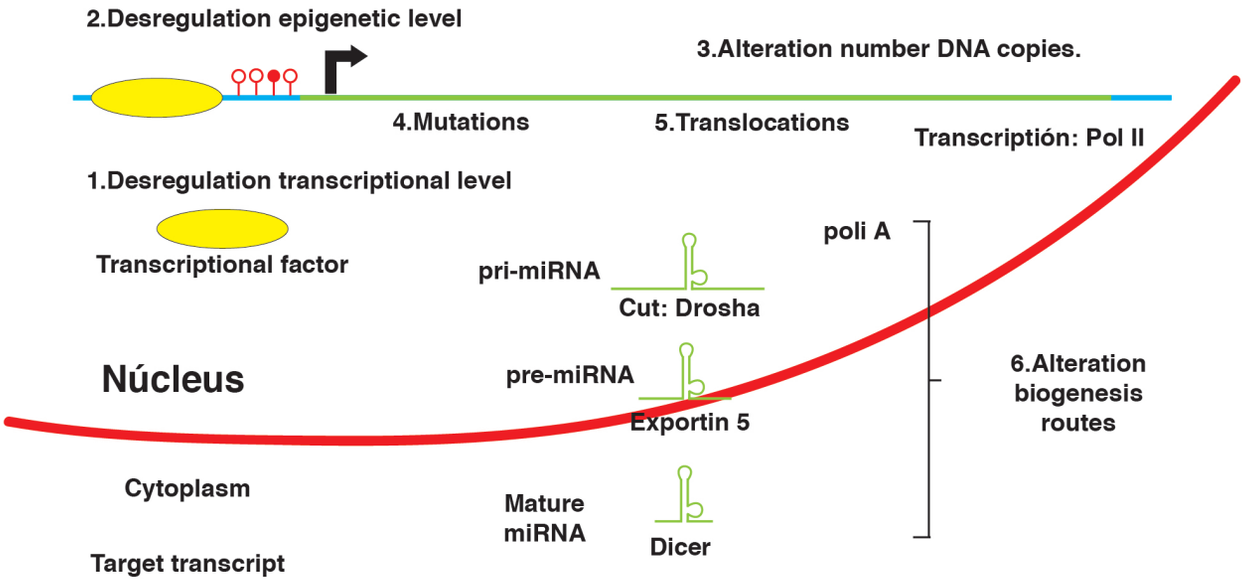

Figure 10. Epigenetic mechanisms regulate the transcription of miRNAs. (A) A CpG island regulates the transcription of an intergenic miRNA. (B) A CpG island regulates the transcription of a gene that harbours an miRNA. (C) An intronic miRNA has its own transcriptional starting point, which is regulated through CpGs. (D) A factor of transcription recruits DNA-modifying enzymes and histones so as to epigenetically regulate a gene that harbours an miRNA which is surrounded by CpGs.

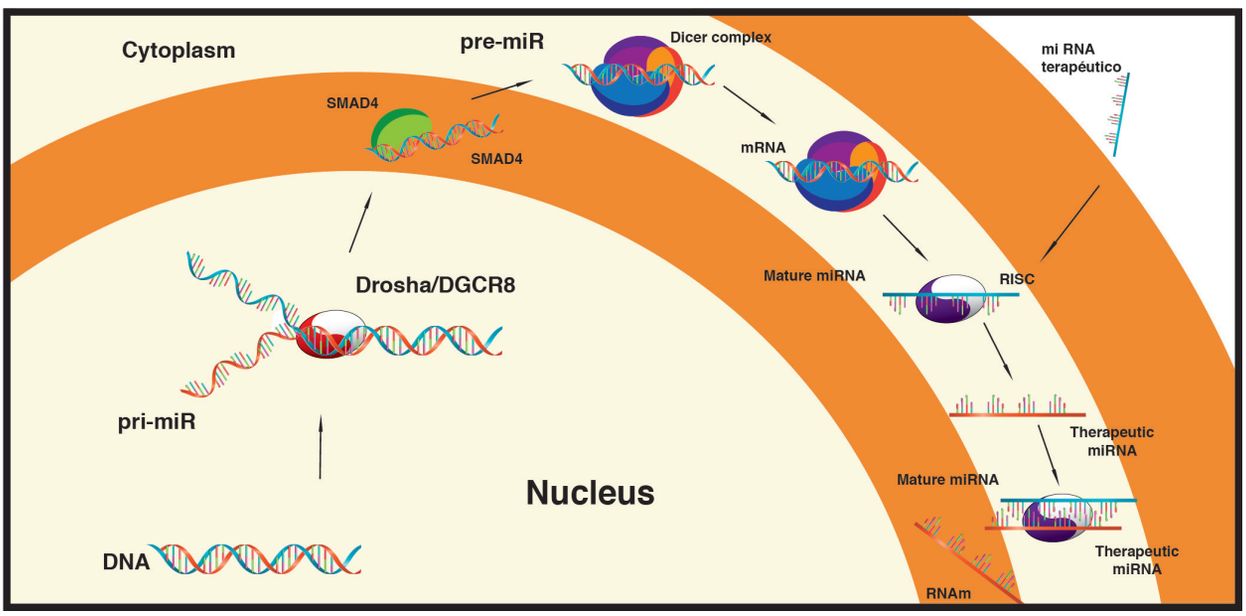

Figure 11. Diverse mechanisms which alter the expression and functionality of miRNAs in human cancer.

Defects in biogenesis of miRNAs. Proteins which participate in the biogenesis of miRNAs may find themselves altered in cancer. In a study that spanned 67 lung cancer patients, a low expression on Dicer1 levels was determined, associated with a poor differentiation of tumour cells and short post-surgery survival [65]. The Argonaut proteins, components of the RISC complex, are in chromosome 1 and are deleted frequently in Wilms' tumours; in

neuroectodermic tumours, an altered expression of these proteins has also been observed. The mechanisms, which alter the expression of miRNAs, are resumed in **Figure 11**.

12. miRNAs as therapeutic targets

Currently, miRNAs are categorised as oncogenes and tumour suppressants in such a manner that a future therapeutic strategy must be headed to inhibiting or activating the altered miRNA, in this sense, in recent years, a therapy of re-expression of microRNAs. The main advantage of miRNA therapy is that its re-expression may influence the expression of hundreds of genes involved in several cell strands and routes. The main obstacle for an effective therapy is the insertion of miRNAs within the cell, because they are molecules that do not freely enter, they are unstable and therefore they may degrade after crossing the membrane of plasma. Another important part is controlling the levels of re-expression of miRNAs to avoid their expression beyond the physiological levels. Another challenge is achieving the antineoplastic agents to cross the haematoma-encephalic barrier. To overcome this inconvenience, different strategies are being developed, such as the intranasal application of oligonucleotides, which is a non-invasive method for the transport of therapeutic agents; unites nucleic acids to cationic lipids, introducing the therapeutic agent by a conjugation with membrane lipids. The *in vitro* studies done with cell strands, antagomiRs, are introduced to cells uniting to their region 5′ a cholesterol molecule; in this way, antagomiR crosses the cell membrane and inhibits the action of the miRNA, sequestering it and uniting by a complementarity of bases, avoiding the inhibition of the target RNAm. Nevertheless, cancer is a complex illness and patients with the same diagnosis may have different genetic and epigenetic alterations and polymorphic variations; therefore, the incorporation of customised medicine is necessary.

In the development of the brain, several microRNAs have been identified with a differential expression profile, for which the differentiation strategy in cancers represents a new approach. There are two focuses on this regard: on one side, there are miRs which favour the growth of the tumour through the inhibition of the cell differentiation, and the maintenance of a small population of tumour stem cells (cells which retain properties of stem cells). In this case, therapies must be directed to these cell under-populations, introducing molecules which block the functions of the miR (antagomiR) [49]. On the contrary, it is known that the overexpression of some miRs such as miR-451 stimulates the CD133+ cells of GBM to differentiate themselves and lose their character of stem cells [67]. MiR-21 regulates several oncogenic routes and strands, for which it participates in the development and progress of gliomas. This makes it a potential therapeutic target in order to treat these tumours. In the same manner, the therapy headed to restore the levels of miR-34a may achieve anti-tumour effects by inducing their differentiation [66]. MiR-124 and 137 inhibit the expression of the RNAm of CDK6, protein CDK6, and they phosphorylate RB in GBM cells, which demonstrate their potential value in treating this illness. Besides, miR-124 and miR-137 have a potent anti-proliferation effect and pro-differentiation effect in GBM CD133+ and CD133− cells [40] (**Figure 12**).

In the following figure, the re-expression of miR-124 is described as a differentiation therapy in GBM.

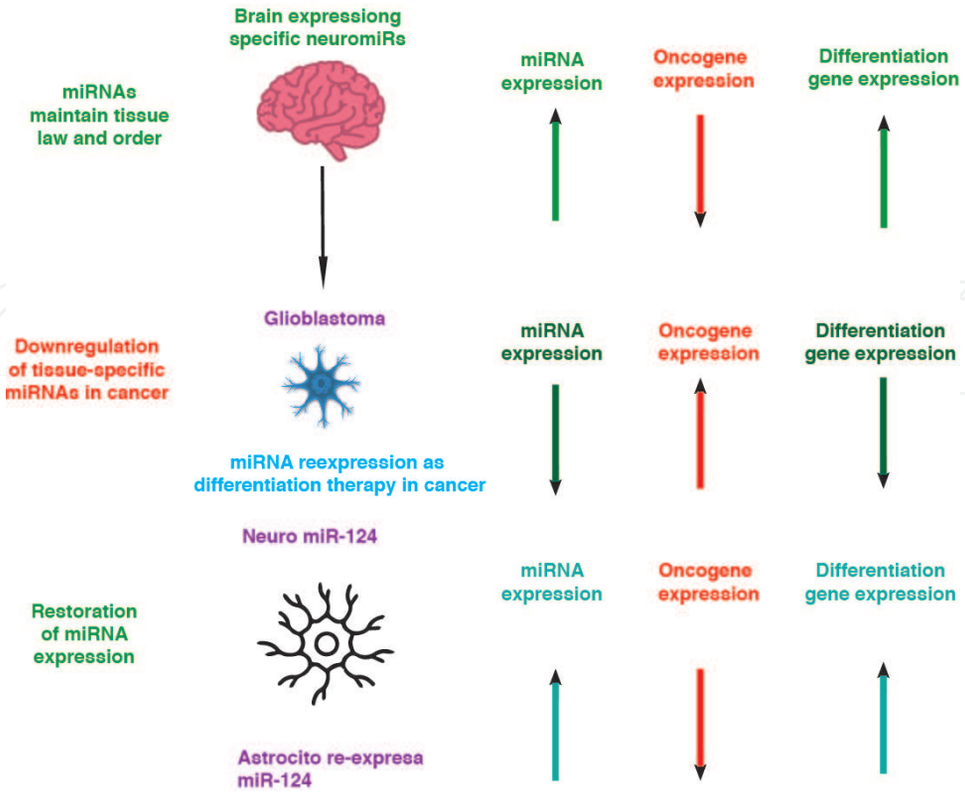

Figure 12. Mature miRNA does not unite to its target RNAm because it is blocked by a complementary therapeutic miRNA.

13. Conclusions and future applications

The expression profile analysis for miRNAs in tumour cells has revealed that the deregulation of these molecules is frequent in a wide array of tumours. MiRNAs may act as tumour suppressants or oncoproteins, which regulate key routes involved in cell growth and apoptosis. Each miRNA may have hundreds of target genes, and several genes are targeted by several miRNAs: this creates a highly complex regulatory network. As we could appreciate in this revision, studies that analyse the expression profile for miRNAs in the different degrees of astrocytomas are scarce; therefore, it is convenient to include a greater number of cases, which helps define the expression profile characteristic for each degree: pilocytic, diffuse, anaplastic and GBM. Within the classing of astrocytic tumours, GBM is the most widely studied tumour, given the fact that it is the most common brain neoplasm in adults and it is quickly disseminated in the adjacent brain tissue, which makes its surgical resection impossible. In GBM, miRNAs participate in several cell processes such as cell proliferation, invasion, angiogenesis and differentiation. Different studies regarding the expression profile of miRNAs in GBM point to overexpressed miRNAs such as miR-10b, miR-21, miR-221 and miR-26 and less expressed miRNAs such as miR-124, miR-128, miR-137, miR-181, miR-7, miR-34 and miR-451. miR-21, miR-221, miR-124, miR-128, miR-181, miR-7 and miR-34 are the best characterised miRNAs with a potential to be used as tumour markers. Nevertheless, it is necessary to correlate the expression profile of miRNAs with clinical and pathological data to answer the therapy or survival of patients.

It is also important to highlight the role that miRNAs undertake in the stem cell, in the differentiation and in cell identity. MiRNAs involved in neural development have also been found deregulated in GBM, which implies that certain miRs allow the growth of the tumour by suppressing the differentiation and maintaining the characteristics of stem cells. Several miRNAs have been identified as having a functional importance in neural development. In particular, miR-7 and miR-124 participate in neural differentiation and are little expressed in GBM. MiR-128 is also altered, but its function in normal cells is unknown. In GBM, the suppression of miR-128 may have severe effects because it may keep the self-renewal of glioma stem cells [42].

The determination and validation of target RNAm will help understand the development of the tumour and will provide potential targets to reduce its growth. In such manner, one of the goals to pursue is to identify a group of miRNAs, whose expression is significantly correlated with clinical parameters and which may be used to classify different degrees of

Little is known about the role of miRNAs as prognosticating indicators. Nevertheless, in astrocytomas, it has been observed that some miRNAs are expressed in a differential manner as miR-221 which is over-expressed in high-grade gliomas, and miR-124 has a lower level of expression in the anaplastic astrocytoma and in the GBM by comparison with low-grade astrocytomas such as the pilocytic and the diffuse astrocytoma. The low expression of miR-137 in astrocytomas is associated with a more advanced clinical phase. The low expression of miR-181b or the high expression of miR-21 was significantly associated with a poor survival of the patient [43].

The miRNAs may have important therapeutic implications, given that they may be functionally antagonised or restored.

MiRNAs are less susceptible to chemical modifications and degradement by ribonucleases by comparison with RNAm. These features of miRNAs allow their detection not only from frozen tissue but also in bodily fluids such as plasma and serum, and even in samples fixed in formol and included in paraffin. This allows for the development of retrospective studies, including a greater number of cases. Particularly speaking, the level of expression starting from bodily fluids represents the most promising advance for a non-invasive diagnosis and allows for their use as biomarkers to detect tumours in early stages and correlating them with clinical development.

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