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Differential MicroRNA Expression in Glioblastoma as a Therapeutic Target or Potential Biomarker

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Abstract

MicroRNA (miRNA) is an epigenetic factor that plays an important role in the post-transcriptional regulation of gene and protein expression. Recent research has shown that in many types of cancer, differentially expressed levels of certain types of miRNA are significantly correlated with the transformation of and ongoing issues caused by cancer cells. Specifically, in Glioblastoma, one of the most lethal and aggressive human cancers, differential levels of miRNAs contribute to the cell's lack of pro-apoptotic gene presence and its high resistance to current treatments. Results from current studies could provide information about which microRNAs are differentially expressed in glioblastoma when compared to normal astrocytes. Differentially expressed microRNAs may be used as a biomarker for diagnosis or a potential therapeutic target for Glioblastoma treatment.

Keywords

MicroRNA, glioblastoma, cancer therapeutics, biomarkers

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Science and Mathematics

Introduction

Function of miRNA

A type of short non-coding RNA of about 20–25 nucleotides, microRNA (miRNA), are found in most eukaryotic cells and certain viruses. MiRNA is primarily involved in the post-transcriptional regulation of gene expression, and they mainly operate by inhibiting mRNA translation or cleaving/destabilizing.

RNA polymerase II transcribes Pre-miRNA, which is then cleaved in succession by the proteins Drosha and Dicer. Once the miRNA is fully mature, it can do two things. First, it can bind with the RNA-induced silencing complex, which orients the miRNA so it can more easily interact with mRNA. When the complex recognizes a specific sequence of nucleotides on the mRNA, it can either directly or indirectly cleave the strand. Second, the miRNA can negatively regulate mRNA through a negative feedback loop or a sort of feed-forward loop.

Due to the abundance of miRNA and the existence of extracellular circulating miRNA— miRNA that is released into body fluids like blood and cerebrospinal fluid— miRNA has the potential to be an effective biomarker for many diseases.

Glioblastoma

Glioblastoma is one of the most lethal human cancers. With less than 5% of patients surviving past 5 years, glioblastoma ranks as the most common of primary malignant central nervous system tumors. Doctors can currently treat the tumor through gross total surgery resection followed by radiotherapy and temozolomide, and tumor-treating fields is a new method proven to extend survival (Gimple et al., 2019).

Poor prognosis is typical for many glioblastoma patients due to the complexity of the tumor. High degrees of intramural cellular heterogeneity, the infiltrative and migratory nature of glioblastoma cells, and a high rate of tumor recurrence all contribute to its poor prognosis. Recurrent tumors also display distinct divergences from the original tumor, which severely inhibits the information obtained from initial biopsies (Gimple et al., 2019). Another contribution to poor prognosis is the presence of differentially expressed microRNAs. Malignancy and stemness-associated miRNAs have been identified in glioblastoma and may regulate genes associated with cancer development and

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radio resistance (Gimple et al., 2019). As a key component in glioblastoma's resistance to modern therapies, miRNAs are ideal targets for the progression of glioblastoma treatment effectiveness.

MicroRNA in Other Cancers

Besides being found in glioblastoma, microRNA (miRNA) also has a distinct role in other cancers. MicroRNA is the key to knowing how cancer stem cells (CSCs), specifically neural CSCs, are created and can ultimately be destroyed because of how CSCs downregulate pro-apoptotic miRNA as compared to normal neural stem cells (NSCs) (Diana et al., 2020). This allows for unregulated growth of the cancerous cells and leads to a multitude of possible cancers. When restored, these pro-apoptotic miRNAs could inhibit anti-apoptotic genes allowing them to act as tumor suppressant miRNAs (Diana et al., 2020).

This kind of information is important to treat multiple different neural cancers. CSCs avoid apoptosis and are nearly immune to most regular forms of treatment (Diana et al., 2020). Neural CSCs can escape apoptosis by the downregulation of miRNAs and avoid other treatments by downregulation of death receptors or anti-apoptotic factors. CSCs including brain tumor stem cells, glioma stem cells, medulloblastoma, neuroblastoma, and melanoma stem cells are the cause of multiple different cancers. (Diana et al., 2020). Although other cancers use miRNAs, the focus in research has been on the neurological side.

MicroRNA and Autoimmune Diseases

MicroRNA plays an important role in the development or regulation of cancer, but recent studies have also shown correlations between the dysregulation of microRNA and the development of autoimmune diseases. Autoimmune diseases are a class of disorders that occur when the body develops an immune response to self-antigens. T cells, part of the immune system, change with the environment and can have a significant impact on autoimmunity. T helper type 17 (Th17) cells release cytokines that activate parts of the immune system such as macrophages and neutrophils (Liu et al., 2018). This increases inflammation and immune response throughout the body. However, there are two types of regulatory T (Treg) cells that have the opposite effect. Naturally occurring Treg (nTreg) cells inhibit inflammation and autoimmunity through cell communication, and inducible Treg (iTreg) cells activate cytokines that also have suppressive properties (Liu et al., 2018). In order to maintain homeostasis in the human body, it is important that Th17 cells and Treg cells are balanced. Upregulation of the gene that codes for Th17 causes increased inflammation and an increased risk of developing autoimmune diseases. Similarly, the downregulation of genes that code for Treg cells also causes increased autoimmunity. The relationship between these two types of T cells is crucial for the human body to function properly.

MicroRNA also plays a role in the gene regulation of these two cell types, therefore impacting their balance and overall autoimmunity. Upregulation of certain microRNAs inhibits tensin homolog and phosphatases, the result highly activated T cells in immune response. MiR-214 and miR-182 are specifically known for having this effect (Colamatteo et al., 2019). MiR-155 seems to play a role in the suppression and activation of cytokines, and studies in mice have shown evidence that downregulation of miR-146 causes both inflammation autoimmunity to increase, as well as an overall suppression of T cells (Colamatteo et al., 2019). Altogether, an upregulation of miRNAs that control the response of Th17 can lead to chronic inflammation and autoimmunity. Similarly,

downregulation of miRNAs that control the Treg response also causes inflammation and autoimmunity to increase. Heightened inflammation and dysregulated immune response lead to development of autoimmune disorders such as rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, and systemic lupus (Liu et al., 2018).

Analysis

MicroRNA Expression in Healthy Astrocytes

MicroRNA (miRNA) plays an important part in maintaining the normal functions of the central nervous system (Sun et al., 2019), and they often have an inhibitory function. Although its role is still not completely clear, an accumulating collection of evidence indicates that miRNA is a key part of many of the roles and tasks of the astrocyte. For instance, miRNA seems to be necessary in the formation of synapses. Research has shown that increased levels of CCL5, a target of miRNA, caused deficiencies in synapse formation (Sun et al., 2019). In addition, this research further supported the theory that miRNA plays a pivotal role in regulating the activation of astrocytes.

When an astrocyte becomes activated, it gains the ability to secrete various signaling substances that regulate neuron development, function, and connectivity (Jovicic & Gitler, 2017). Among these substances are exosomes carrying miRNA shown to be significantly different from the miRNA that remains inside the cell. The miRNA carried by exosomes provide a mechanism for differentiating the function of various miRNAs within the cell. Also, while not much is yet known about this exosomal miRNA, recent evidence shows that miRNA plays a key role in the inflammatory response, diminishing the activity of target neurons and downregulating the transcription of proteins required for neuronal excitability (Chaudhuri et al., 2018). In fact, miRNA likely plays a major role in the neurogenic stress response (Luarte et al., 2017). For this reason, certain miRNA could be a good biomarker for central nervous system inflammation and stress points (Lafourcade et al., 2016).

Finally, there is no one fast and easy way to tell what a healthy expression of miRNA in astrocytes is since the answer will depend on many factors, such as age and anatomical location. Researchers find a high expression of miRNA in the fetal germinal matrix, likely for developmental purposes (Rao et al., 2016). There is a higher expression of miRNA in adult white matter than in fetal white matter, while in grey matter the two seem comparable. Also, similar overall levels of miRNA exist between adult white and grey matter; however, different levels of various miRNA appear to be expressed in each (Rao et al., 2016).

MicroRNA Expression in Glioblastoma

MiRNAs have a close relation to the biological features of the glioblastoma stem cells (GSCs) and predict the survival in glioblastoma patients. Fifty-one miRNAs are associated with glioblastoma's stem-like phenotype, and nine were identified to be strongly upregulated in GSCs: miR-9-3p, miR-93-3p, miR-93-5p, miR-106b-5p, miR-124-3p, miR-153-3p, miR-301a-3p, miR-345-5p, and miR-652-3p (Sana et al., 2018). Both miR-9-3p and the hairpin counterpart miR-9-5p are expressed in the brain. These miRNAs affect the Notch signaling pathways, which surprisingly promotes differentiation of neural stem cells. Furthermore, this affected Notch pathway appears to induce

miR-9/9*. (An asterisk is added to the name of the miRNA strand to denote the star strand.) Abundant in CD133+ GSCs, these miRNAs seem to contribute to glioblastoma's high resistance to modern therapy (Sana et al., 2018). The cluster containing miR-106-5p, miR-93-5p, and miR-93-3p seems to have a close connection to the biology of stem cells. Research shows the inhibition of this cluster in CD44+ gastric cancer stem-like cells suppresses the TGF-ß/Smad pathway (Sana et al., 2018). MiR-153 was shown to have a high expression in GSCs, and its overexpression reduced GSC's tumorigenic capacity (Sana et al., 2018). Of the nine upregulated miRNA, miR-652, miR-345, and miR-9* all positively contributed to a higher risk score and poorer prognosis, while miR-301, miR-153, miR-93, and miR-106b negatively contributed (Sana et al., 2018). These results signify that miRNAs are closely involved in the biological characteristics in GSCs, making them ideal targets for therapeutic treatment.

MicroRNA Expression as a Biomarker

The increase of certain microRNA strands allows them to be used as a biomarker by comparing their levels in the supposed cancerous tissue to levels in a known normal tissue of the same type. More research is needed until miRNA can act as a useful and reliable biomarker. The benefits of uncovering this diagnostic method are undoubtedly great as evident in breast cancer (BC), which already has research into its microRNA being reviewed as a possible diagnostic tool (Adhami et al., 2017).

For miRNA to be used as a biomarker, there must be actual upregulation and downregulation of the different strands in cancerous tissue when compared to normal tissue. Studies show there are around 144 different miRNAs found with 74 being upregulated and 70 being downregulated (Adhami et al., 2017). In total, there are at least 30 differentially expressed miRNA across multiple studies. These were all expressed in a constant direction (Adhami et al., 2017) making them useful for use as a biomarker.

Using this kind of detection shows great promise for modern day medicine. The current mammography technique is useful for catching early-stage breast cancer, but the technology is still unreliable and can lead to false positives as well as overdiagnosis and subsequent overtreatment of some minor cases (Adhami et al., 2017). And then there are those that do not get caught at all and are thus never treated. Using this new biomarker diagnosis via miRNA could help catch false positives or false negatives before any major harm is done. There are also a few miRNAs, specifically MiR-21, which have altered expression in many different types of human cancer (Adhami et al., 2017).

Despite the potential of this study, there are still some drawbacks to using this kind of diagnostic marker. The first is the wide range of inconsistency found between research for most biomarker targets (Adhami et al., 2017). Second, researchers would need to compile a large library of all the consistently different regulations in order to be the most effective and accurate. Generally, the subject needs more research because of the major inconsistencies among studies and reviews regarding miRNA and its regulation (Adhami et al., 2017).

MicroRNA as a Therapeutic Target

MicroRNAs and their expression in glioblastoma cells may serve as a potential therapeutic target to treat this aggressive form of brain cancer. An overall lack of balance between tumor suppressor

miRNAs and oncomiRs contributes to the progression of various cancer types, and current researchers search for ways to correct the expression of certain microRNAs to possibly treat or slow the progression of cancer types. By understanding which miRNAs are differentially expressed in glioblastoma tumors and discovering how to control the expression of certain miRNAs, it may be possible to minimize or even reverse the effects of glioblastoma and lower the fatality rate of this type of brain tumor.

When tumor-suppressing microRNAs lose function, malignant tumors can form. As previously discussed, microRNAs are responsible for regulating gene expression, and cancer research primarily focuses on the expression of tumor suppressor proteins and proto-oncogenes. Tumor suppressor microRNAs act by inhibiting the mRNAs that code for oncogenes. In many malignant tumors, tumor suppressor miRNAs have significantly lost their function through mutations, epigenetic silencing, or mistakes in mRNA processing (Mollaei et al., 2019). Some examples of a loss of function of tumor suppressor miRNAs in malignant tumors are miR-449a and miR495. MiR-449a is known to be downregulated in glioblastoma cells and may be contribute to the aggressive growth and multiplication of these cells. MiR495, on the other hand, is known to be downregulated in breast cancer, prostate cancer, and leukemia (Mollaei et al., 2019). Among many others, these downregulated miRNAs play a role in the development and progression of various cancer types.

Another type of miRNA that plays a role in the development of cancer types is oncomiRs. OncomiRs are typically upregulated in cancerous tumor cells because of mutation or misregulation of certain pathways such as histone methylation or promoter methylation (Mollaei et al., 2019). Common miRNAs that are upregulated in cancer cells are miR-155 and miR-21. MiR-155 is overexpressed in lung cancer, breast cancer, and multiple forms of lymphoma, while MiR-21 is overexpressed in almost every type of human cancer—including blood and organ cancers (Mollaei et al., 2019). While little is known about the effects of upregulated miRNAs, it is clear they are important to the development of cancer like tumor suppressor miRNAs are.

Understanding the expression of miRNAs and their role in cancer development could lead to development of a treatment for some of the most lethal cancers, such as glioblastoma. Theoretically, upregulating the downregulated tumor suppressor miRNAs or downregulating the upregulated miRNAs could recreate a balance between tumor suppressor proteins and oncogenes. Two current theories exist to explain how this information could be used in a viable treatment: miRNA reduction therapy and miRNA restoration therapy.

MiRNA reduction, also called inhibition therapy, is based on the concept of inactivating upregulated miRNAs. Several methods are currently being used to try to meet this end, including miRNA sponges, locked-nucleic-acid antisense oligonucleotides, anti-miRNA oligonucleotides, miRNA nanoparticles, antagomirs, and multiple-target anti-miRNA antisense oligodeoxyribonucleotides (Mollaei et al., 2019). The basis behind all these methods is the same: to inhibit the expression of oncomiRs and slow the progression of various cancer types. However, there are some challenges related to this therapy. For example, many oncomiRs have multiple targets and focusing on limiting one miRNA at a time would not have a noticeable effect on cancer in a clinical setting (Mollaei et al., 2019).

The concept behind the other method, miRNA restoration therapy, is to upregulate the tumor suppressor miRNAs and allow them to slow the production of oncoproteins within cancerous cells. The goal of this kind of therapy is to have cancerous cells take up microRNA genetic material that

will prevent the cells from continuing to grow and even cause an increase of apoptosis (Mollaei et al., 2019).

After discussing the strategies behind using miRNAs to treat cancer types, it is important to understand how these theoretical therapies could be integrated into clinical medicine. Currently, biomedical researchers are investigating four main methods of drug delivery: anti-miRNA nucleotides, viral delivery, nonviral delivery, and small molecule drugs (Lee et al., 2020).

The first type of delivery is the anti-miRNA nucleotides. RNA molecules are made more chemically stable by altering their sugar-phosphate background, which makes them less susceptible to degradation within the cell. By making RNA molecules more stable, certain nucleotide sequences could be synthesized and used to create anti-miRNA oligonucleotides that decrease the levels of specific miRNAs. Studies in mice have shown that this RNA modification can be successfully used to target a certain miRNA and decrease its expression within the cell in vivo (Lee et al., 2020).

The second delivery strategy, viral delivery, has been studied for years as a mechanism to edit genes and change gene expression. In the case of cancer treatment, viruses can be used to control gene expression and limit harmful side effects, and viruses may also be able to remove certain destructive genes completely. For instance, a drug called Luxturna uses viral delivery to alter gene expression and treat Huntington's disease by downregulating the Huntington gene (Lee et al., 2020). While this has not been used in clinical settings with cancer, it shows promising results as a therapeutic treatment.

Nonviral delivery, the third method for delivery of treatment, includes polymeric vectors, lipids, and both inorganic and RNA nanoparticles. The goal of nonviral delivery is to allow the body to take up the drug without eliciting any immune response or causing the miRNAs to become completely degraded (Lee et al., 2020). The nonviral delivery methods described above have been proven to be safer for patients when compared to viral delivery, minimizing harmful side effects.

The final delivery strategy that could be used to target miRNAs is the use of small molecule drugs. These drugs can be used to target specific miRNAs and transcription factors, changing the level of expression by inhibiting or inducing them (Lee et al., 2020). Small molecule drugs appear to be safe and effective for clinical use and may be able to help regulate the miRNA expression in a way that slows cancer progression in patients.

Conclusion

Summary of Findings

Based on the way microRNAs (miRNAs) are upregulated or downregulated in cancerous tumors, specifically glioblastoma, there are two types of therapy that are being explored as potential treatments—miRNA reduction therapy and miRNA restoration therapy. MiRNA reduction therapy targets upregulated miRNAs and downregulates them in cancer patients to slow the progress of malignancies. MiRNA restoration therapy theoretically upregulates downregulated tumor suppressor miRNAs to also help slow cancer progression.

Biomarkers can be used for diagnosing cancerous tumors. This is done by finding the specific miRNAs that are upregulated and downregulated in comparison with normal health brain tissue.

Using this sort of diagnostic can help reduce testing when a tumor can either be confirmed or ruled out.

Potential Limitations

As mentioned previously, there are a few limitations to the therapies that are currently being researched. First, it would be difficult to create a miRNA targeting treatment that the human body will accept; otherwise, the immune system would attack the treatment. Second, targeting one or two miRNAs would have minimal effect on cancerous tumors, and upregulating or downregulating a large number of miRNAs has proven to be challenging.

Furthermore, when using them for detection, finding biomarkers in any patient is extremely difficult. Because multiple different markers exist, researchers would also need to compile a large library of marker types and compare those markers across healthy and cancerous tissue. Until these things are done, this detection method cannot be considered viable.

Further Research Needed

To use miRNAs as a potential treatment for glioblastoma, much additional research is needed. Aside from arranging clinical trials and developing a treatment that is safe and effective for clinical use, it is important to know exactly which miRNAs are impacted by glioblastoma and what their regulation patterns are. Researchers would also need to gain a better understanding for what microRNAs cause glioblastoma and which microRNAs are affected as a result of glioblastoma. Additional research on these topics is crucial to creating the most effective treatment.

Many more tests of potential biomarkers also need to be done for the effective use of biomarkers. As mentioned, a large library of biomarkers must be built up. Then, researchers need to find consistency in each type for them to be used as biomarkers for possibly presenting a cancerous tumor. Once consistency is established, scientists must determine how to administer the tests in the least invasive, most reliable, and most efficient way possible. Then, the full potential of the biomarkers can be used to help detect the cancer while it is still treatable.

Developing a broad understanding of microRNAs and the role they play in autoimmune disorders and cancer, specifically neural cancers, can lead to advancements in the field of molecular biology research. MicroRNAs were recently discovered, and there is still much to learn about their expression, purpose, and limitations as it relates to various diseases. As more experimental data emerges, the hypotheses that scientists now have regarding microRNAs may be accepted or changed, shaping future research, treatments, and diagnostic procedures.

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