Recent Blood and Intracranial Molecular Biomarkers to Moderate Depression and Major Depressive Disorder

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Abstract. Depression, especially moderate depression and MDD, brings huge pressure and loss to patients and society through a series of symptoms. However, the current diagnosis of depression is short of objective, quantifiable, and absolute detection techniques. If we can find specific depression molecular biomarkers and high-sensitivity and high-accuracy molecular biomarker detection technology, it can help patients with depression to diagnose and predict earlier. In this paper, the heterogeneous biomolecules of depression are classified into intracranial markers and blood markers, and the biomolecules with representative value are listed. Meanwhile, it analyzes and compares the current popular biological detection technologies, IHC and RNAscope. If the heterogeneous depression biomarkers can be detected by the combination of IHC and RNAscope technology, the accuracy and timeliness of depression diagnosis will be greatly improved, and lay the groundwork for better depression treatments in the future.

Keywords: Molecular Biomarker, Moderate Depression, Major Depressive Disorder.

1. Introduction

Depression, as a mental illness, can cause patients, especially those with moderate and severe depression, to have a high tendency to self-harm and suicide. Depressed patients have abnormalities in the part of the brain structure related to emotions compared with normal people, such as the volume changes of the prefrontal cortex (PFC), hippocampus as well as neuronal activity and glial damage in patients[1]. This causes great pain to patients both physically and psychologically, and has a great impact on their life and work, and also brought great economic burden and social pressure.

The subjects of this study were moderate and severe depression. Some evidence suggests that whether the depression is serious may be related to levels of neurochemical metabolism[2]. PFC has a huge impact on the regulation of human emotions. So, that is the reason why the authors assessed the severity of depression based on the level of neurochemical metabolites in the prefrontal cortex (PFC)[3]. Some investigators have found that the concentrations of neurochemical metabolites in patients with moderate depression are not significantly different from those in normal subjects, while NAA/Cr levels in MDD patients are significantly less than those in controls, and through proton magnetic resonance spectroscopy (1 H-MRS), it was found that some brain regions of MDD patients had structural and functional abnormalities, including the PFC region, hippocampus and some ganglia[4].

Therefore, if depression can be diagnosed as soon as possible at an early stage, and psychological intervention and treatment can be carried out in time, the pain and treatment costs of patients can be greatly reduced. However, despite the fact that depression is very common worldwide, there is still a lack of a quantifiable and directly detectable method for the diagnosis of depression. Compared with other diseases, such as cancer and diabetes, the detection and diagnosis of these diseases can be more objective, quantifiable and accurate by finding specific small molecule biomarkers and using corresponding biosensors. However, when it comes to the current diagnosis of depression, it is more often diagnosed by detecting the patient's concentration time, sleep conditions, and psychological test questions. These methods are relatively subjective, and at the same time are very complicated to operate, which can easily lead to misdiagnosis and delay the disease. Therefore, a more objective, quantifiable, and rapid diagnosis of depression is urgently needed. This article sorts out the

intracranial and blood biomolecular markers that scientists have discovered in recent years that may serve as depression, and summarizes and compares them. At the same time, IHC and RNAscope two biological detection techniques are analyzed and compared.

2. Intracranial Biomarkers

The classification of intracranial biomarkers mainly refers to the effect of these biomarkers on the brain and spinal nerves. In addition, the acquisition method for detecting small molecules is more in serum or buccal cells.

2.1. Neurofilament light protein

Many experimental studies have shown that axonal damage may be caused by depression [5]. And the accepted biomarker of axon damage may be a neurofilament light protein measured in serum and cerebrospinal cord[6]. Neurofilaments are nonspecific biological fluid markers, which are cylindrical proteins located in dendrites, somatic cells, and especially neuronal axons[7]. It can promote the structural stability of nerve growth, promote axonal growth, and promote intracellular transport. However, neurofilament levels are significantly higher than the normal range due to any type of axonal injury like inflammatory or degenerative[8]. Therefore, the level of serum neurofilament protein can be used to quantify the extent of axonal damage, which can also be used for the diagnosis of depression[9]. In the case of a diagnosis of depression, regularly measuring the level of neurofilament light protein timely and the subsequent severity can provide a basis for judgment in later treatment[10].

Studies have shown that after reaching the interstitial fluid, neurofilament light protein can be detected in the cerebrospinal fluid, and it can be detected in serum at a concentration of about 40 times lower, so it will have a certain impact on the accuracy of the detection[11]. What's more, not only depression can cause axonal damage, but also different neurological diseases such as Alzheimer's disease, may lead to different levels of neurofilament protein[12]. Therefore, due to the complexity of the etiology, a single detection of neurofilament light protein level may lead to misdiagnosis[11]. Besides, the quantitative detection of neurofilament protein requires a lot of cost and time, which greatly limits the clinical practical use of this diagnostic method.

2.2. Histone Deacetylases

Acetylation and deacetylation can be controlled by histone lysine (Lys), which will affect the replication, transcription and repair of DNA as well as the cell cycle progression. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) can regulate the balance of histone deacetylation levels and promote the regulation of cellular functions [13]. The levels of histone regulation by HAT and HDAC can reflect the state of stress-induced depression and the effects of antidepressant therapy[14].

Additionally, scientists found that the expression of HDAC affects the expression of brain-derived neurotrophic factor (BDNF)[15]. BDNF is a key ligand that directs ongoing neuroplastic processes required for neurodevelopment and behavioral adaptation, and can be used to slow neuroinflammation while contributing to the development of the nervous system[15]. Through various experiments, it was found that the concentration of BDNF in most patients with depression was significantly lower. Therefore, it is proved that histone acetylation is a potential diagnostic biomarker and therapeutic target for detecting depression. At the same time, it was also found in the experiment that the activity of HDAC will be affected by gender, which also confirms that the prevalence of depression syndrome is related to sex differences[14].

2.3. MORC1 methylation

The MORC family CW-type zinc finger 1 (MORC1) gene has been confirmed to be closely related to major depressive disorder. In addition, it is found that MORC1 methylation is also closely related

to early life stress (ELS)[16]. To studying MORC1 methylation by extracting DNA from buccal cells, we found that increased DNA methylation around the MORC1 promoter was significantly associated with major depressive disorder. However, the methylation of MORC1 after ELS showed a decreasing trend[17].

This suggests that the experimental results of birth complications are inversely correlated with methylation, making MORC1 not clear in demonstrating the relationship between ELS and MDD[18]. However, this confirms that MORC1 is a stress-sensitive gene with the potential to be the biomarker of depression. [19]. But because DNA methylation varies in different tissues, MORC1 methylation studied using buccal cells are not enough to represent the DNA methylation signature in the tissue (the brain) we should focus on[16].

2.4. The inhibitory neurotransmitter gamma-aminobutyric acid

The study in this article was based on a population of adolescents with MDD[20]. Prior to this research, numerous studies have linked dysregulation of the main inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the brain to the pathophysiology of major depressive disorder[21]. The researchers used linear regression models to determine the relationship between GABA concentrations and MDD severity by collecting data on GABA levels of adolescents who underwent magnetic resonance spectroscopy (MRS) scans 9 months earlier, with follow-up surveys[20].

Although this is only a preliminary study, the analysis and research on the level of GABA further confirmed the relationship between the imbalance of GABA in the brain and MDD, which has certain research value for the timely diagnosis of major depression in adolescents[20, 22].

3. Biomarkers in blood

Different from intracranial biomarkers, the mechanism of action of biomarkers in blood lies more in the immune inflammatory response of the central nervous system caused by depression in patients.

3.1. Serum NLRP3 Inflammasome

In recent years, scientists have been studying the immune inflammation triggered by depression[23]. Innate immunological responses such as infection, inflammation, and autoimmunity are mediated by NLRP3, an intracellular multiprotein complex. In a research report, it was stated that the level of NLRP3 protein in peripheral blood mononuclear cells of patients with depression was higher than that of non-depressed patients[15]. Thus, there is a relationship between the NLRP3 inflammasome and mild to moderate depressive symptoms, according to preclinical studies.

Aside from the fact that NLRP3 can be used to diagnose depression, researchers discovered that serum NLRP3 levels in the reactive depression group were considerably lower than those in the endogenous depression group and the healthy group[24]. Therefore, the concentration of serum NLRP3 inflammasome can also be used to distinguish reactive depression from endogenous depression, which can provide targeted treatment for different patients[15]. However, the study was only appropriate to people with mild to moderate depression, according to the article. At the same time, it was found during the experiment that after controlling for covariates, the combined dependent variable between the groups did not show any significant difference, which greatly reduced the accuracy of detection[15].

3.2. Vasopressin surrogate marker copeptin

In drug-naive individuals with severe depressive illness as well as the comparison of the in vivo concentrations after receiving drug treatment, the baseline and dynamic levels of plasma CoP, plasma ACTH, and cortisol (CORT) were measured. The researchers found that the amount of CoP in the body of the drug-naive patients significantly higher, while its concentration level was statistically inversely correlated with the treatment effect[25, 26]. However, other baseline plasma ACTH and cortisol (CORT) levels were not associated with major depression[27].

Through this preliminary study, it was confirmed that the level of CoP in blood may serve as an endocrine biomarker of antidepressant response[28]. However, because the mechanism of action of MDD and mild to moderate depression is very different, the results of this study are only applicable to the diagnosis and treatment of severe depression[25].

3.3. Serum hepatocyte growth factor

Serum hepatocyte growth factor (HGF) is a heterodimeric glycoprotein of 82kda, 674 amino acid residues. It mediates the inflammatory response to tissue damage and regulates the growth, cell motility, and morphogenesis of a variety of cells[29]. HGF signaling has also been found to have anti-inflammatory, anti-fibrotic, and pro-regenerative activities in various types of tissues. Except for this, scientists have discovered that it plays an important role in the development of the central nervous system and has a potential link with neurological diseases.

The HGF level of depressed individuals was significantly lower than the normal level, and it can be confirmed that HGF level is directly related to the severity of depression. Because some studies have shown that HGF is involved in the regulation of GABA, particularly through anxiety-suppressing activity. So, scientists speculate that this may be due to the reduction of HGF levels in depressed patients, which leads to a decrease in GABA in the body[30]. At the same time, low concentrations of GABA may trigger anxiety, which also explains the symptoms of anxiety in some depressed patients.

4. Detection technologies

4.1. IHC

The traditional operation method of Immunohistochemistry (IHC) technology is to observe the target antigen in tissue sections or cells by fluorescence microscope or other observation means on the basis of identifying specific antibody-antigen[31]. Through this microscopy-based technique, IHC can obtain effective information about protein expression in tissues at the single-cell or subcellular level while maintaining the integrity of the cellular or tissue structure[23]. Even if there are only a small number of cells in the identified sample, such as tumor cells or stem cells, it is still possible to obtain the useful information needed. This is a great leap and breakthrough compared to traditional tissue detection methods[32]. The technique was first discovered in the 1940s, with the development and widespread use of peroxidase-labeled antibodies, allowing IHC to detect routine tissue sections and living cells, such as FFPE (formalin-fixed, paraffin-embedded) tissue. In the early 1990s, IHC was gradually applied in clinical medicine and diagnostic pathology[33]. At present, IHC technology is widely used in the research of various biological or medical experiments.

Before IHC was widely used, most of the other techniques were difficult to be applied clinically due to the difficulty of DNA preservation and the easy interference of target cells by other tissue cells[34]. With the development of IHC technology, it plays an increasingly important role in clinical medicine, especially in the detection and treatment of cancer[35]. In the research of thyroid cancer, IHC technology plays an important clinical application[36]. Papillary thyroid cancer (PTC) is a common thyroid malignancy. Activating mutations in the mitogen-activated protein kinase/extracellular signal-regulated protein kinase pathway are the most common gene mutations in PTC, of which BRAF V600E mutation (BRAF+) is the most common. Scientists have discovered a specific antibody, which enables observations with IHC technology with greater sensitivity and specificity, and becomes a very viable method to detect the BRAF V600E mutation in PTC[37]. At the same time, it has also been confirmed that the ability of IHC technology to quantify mutation expression may be more reliable and predictable than molecular sequencing. In the study of breast cancer, IHC techniques have shown higher sensitivity than traditional histology in detecting micrometastases[31]. Several studies have shown that immunohistochemistry of sentinel lymph nodes in breast cancer is beneficial for the detection of micrometastases[35]. However, in experiments, it was found that the IHC detection technology may produce false negatives when detecting patients

with poorly differentiated breast cancer. In the meantime, through the study of lung cancer, IHC for the antibody of EML4-ALK fusion protein provides a widely available detection method for the study of lung cancer cells[23]. Although IHC cannot completely replace the expensive FISH analysis technique, it can still be a very effective screening tool. In detection, the use of cost-effective and relatively fast IHC technology can prevent unnecessary waste when using FISH technology[33].

4.2. RNAscope

In molecular diagnosis based on RNA ISH, RNA is easily degraded, which greatly limits the development of RNA ISH technology[36]. The RNAscope technology, as an improvement of the RNA in situ hybridization (ISH) method, is a quantitative in situ gene expression measurement technology[38]. RNAscope detects cell-targeted RNA-specific signals, as well as partially degraded RNA[39].

Proteins, DNA and RNA as biomarkers are important clinical diagnostic and predictive tools[37]. Detection of cancer gene expression relies on a number of cancer-related RNA biomarkers. Since the detection of RNA biomarkers is susceptible to signal interference from various other cell types, the use of whole tissue homogenates for the analysis of specific RNA biomarkers is error-prone[40]. However, using tissue-destroying methods to obtain cell-targeted RNAs will lose the spatial and specific information about cells. Due to the particularity of RNAscope technology, it is possible to preserve tissue morphology in FFPE tissue sections, thereby preserving the integrity and spatiality of tissue structures[37]. Therefore, RNAscope technology can be used for specific cells or tissues. Recently, most of RNAscope research is focused on cancer diagnosis and treatment[39].

In breast cancer research, RNAscope technology can be used for screening of mammary gland whole-mounts, and through the detection of formalin-fixed and paraffin-embedded (FFPE) tissues, lesions in the breast region can be quickly and accurately identified. This detection method can quantitatively identify and evaluate the whole-mounts for morphologies during various breast pathologies and carcinogenesis, which is helpful for the diagnosis of the disease and the deployment of treatment. In the research on bladder cancer, it was found that urothelial carcinomas (UCs) account for more than 85 percent of bladder cancers[38]. However, UC is particularly prone to differentiate, which may lead to variation in various tissues. Because the morphological features of the variants differ from those in traditional UC, this makes the origin of UC urothelial variants difficult to identify, especially when the variants are present in metastases. Luckily, RNAscope has high accuracy and sensitivity for the detection of UPK2 in UC, and it has the potential to prevent false negative results in the detection status of UPK2. This enables RNAscope technology to play a huge role in identifying UC variants. In the research of lung adenocarcinoma, scientists found that TTF-1 and napsin A are biomarkers to differentiate between lung adenocarcinoma and lung squamous cell carcinoma in the immune system, and play a vital important role in identifying primary lung adenocarcinoma of unknown origin effect[37]. In the clinic, experiments have shown that RNAscope has extremely high accuracy and sensitivity in detecting TTF-1 and napsin A expression in targeted cells in primary lung adenocarcinoma. At the same time, the operation of RNAscope test is relatively simple and has high repeatability, which greatly improves the reliability of case diagnosis.

4.3. Comparison and Analysis

By comparing the above two detection technologies in the experiment, we found that IHC and RNAscope technology have a high correlation[32]. However, compared with the newly discovered RNAscope technology, IHC has shown great limitations. The biggest limitation is that the accuracy of IHC is affected by the density of targeted cells and tissues and the sensitivity limits of the detection system[36]. Taking the detection of breast cancer as an example, when immunohistochemistry (IHC) is used in clinical experiments to determine the disease state of patients, it is found that this method is highly subjective, requires a large amount of tumor tissue, and is prone to false negative results. When using RNAscope technology, it can provide quantitative data and can effectively avoid false negative results.

The increased sensitivity and specificity of RNAscope technology is due to the use of a unique double-Z probe strategy ("double-Z" or ZZ). The ZZ pair of the complementary regions are designed to hybridize to the target RNA. When hybridized to the target RNA, a sequence which is complementary to the preamp sequence will be formed by each ZZ pair, allowing simultaneous background suppression and signal amplification[39]. It also enables single-molecule visualization, preventing signal amplification at non-specific sites, allowing detection of single molecules under standard fluorescence or bright-field microscopy. The current laboratory application further expands the application of RNAscope detection technology in pathological diagnosis and differential diagnosis. Especially for a set of highly specific, diagnostic IHC markers, RNAscope can detect some specific, organ-specific biomarkers, and may also serve as another example in the event that IHC fails to show a detectable signal[36]. Therefore, considering the heterogeneity of individual tissues, IHC can be used in combination with RNAscope technology.

Another noteworthy is that RNAScope is a meaningful technical discovery of RNA ISH methodology in the field of neurobiology. This technique can present many advantages in neurology. Several experiments have shown that it is highly compatible and highly sensitive to conventional formalin-fixed, paraffin-embedded fetal brains[36]. Not only does it have a significant background suppression effect, it can also detect genes expressed at low levels, such as receptors and morphogens. This means that it has great potential to be used in the diagnosis and detection of depression biomarkers.

5. Conclusions

In summary, the study of molecular-level quantifiable biomarkers of depression remains of great medical value. These years researchers have found a large number of possible molecular biomarkers, as well as very potential detection technologies for heterogeneous biological small molecules, which will further promote the research and diagnosis of depression at the molecular level. However, there are still some limitations in this paper. The classification of biomarkers for depression in this paper cannot summarize all the existing types of biomarkers. Besides, the small molecule biomarkers listed in this paper are not absolutely comprehensive. Additionally, IHC and RNAscope detection technology are now more used in the pathological diagnosis of cancer, but not widely used in the detection of small molecular markers of depression. In the meantime, this paper also found that there is a great correlation between the pathological mechanisms of many small molecular markers, but the mechanism and connection still need further research and experiments.

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