miRNAs as Biomarkers for Traumatic Brain Injury

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microRNA (miRNA) profiling has attracted increasing interest due to these molecules' ability to regulate physiological and pathological processes. The evidence of differential miRNA expression in both animal models and human samples of traumatic brain injury (TBI) has laid the basis for comprehension of the underlying pathophysiological mechanisms, thus allowing us to identify some of them as possible TBI diagnostic biomarkers.

traumatic brain injury | miRNA | forensic | autopsy | biomarkers

1. Traumatic Brain Injury

Traumatic brain injury (TBI) is an impairment of cerebral functions due to direct or indirect mechanical insults, such as blasts, assaults, collisions, penetrative injuries, etc. It can be classified as a primary or secondary injury; the first one consists of the acute pathological changes induced by an external force at the (time) of the impact, which are mainly represented by internal hemorrhages, brain contusion, and axonal and vascular damage; the second one comprises all those pathological processes leading to further impairment of the neurological functions (oxidative stress, glutamate excitotoxicity, Ca^{2+} overload, inflammatory response). With an annual incidence of around 50 million individuals, TBI represents one of the leading causes of disability worldwide since the related neurodegeneration increases the risk of developing chronic behavioral, cognitive, and physical impairment, as well as dementia, Alzheimer's disease, and Parkinson's disease [1][2][3][4][5].

TBI severity can be classified as mild, moderate, or severe according to the Glasgow Coma Scale, a scoring system that consists of the evaluation of eye, motor, and verbal responses; a score of 13–15 corresponds to a mild TBI (mTBI); a 9–12 score corresponds to a moderate TBI; and a ≤8 score identifies a severe TBI. Other parameters contributing to better defining the severity and prognosis of TBI include clinical outcome, loss/alteration of consciousness, impaired mental state, and brain alterations detected by imaging [1][5].

From a diagnostic point of view, imaging represents the gold standard. Computed tomography (CT) allows the identification of the injured cerebral areas and the extent of injury, thus guiding the most appropriate clinical or surgical management. In selected cases, MRI can also provide important information due to its better tissue contrast and increased sensitivity compared to CT. Nonetheless, mTBI is frequently associated with a lack of visible signs of head impact (bleeding, lacerations) on neuroimaging, thus making it difficult to either achieve a correct diagnosis or make a prognostic evaluation [1][2][3]. Therefore, to overcome such limits, due to the knowledge of the pathophysiological mechanisms underlying TBI (release of cytokines, chemical mediators, and

neurotransmitters; NO-dependent and Ca^{2+}-dependent induction of apoptosis), several researchers have engaged over time in the identification of fluid biomarkers of injured axons, neuronal and glial cells, as well as inflammation biomarkers [3][6].

Since almost 70% of miRNAs are reported to be expressed in the central nervous system—where they guide all stages of neurodevelopment and function—those involved in the molecular pathways activated by TBI have been gaining attention over the last decade as possible fluid biomarkers [3].

The complex TBI-related molecular network in which soluble miRNAs are involved was carefully elucidated in a meta-analysis by Cente et al. [5], who made use of bioinformatic systems to identify the target genes for deregulated miRNAs isolated from plasma, serum, and cerebrospinal fluid, and the related signaling pathways, to link severe TBI to the pathogenesis of neurodegeneration. As a result, they found that the miRNA-targeted genes following severe TBI were involved in a great variety of processes, such as brain development, neurogenesis, myelinization, oligodendrocyte differentiation, regulation of synaptic plasticity, axon guidance, and regulation of inflammatory genes. As for the molecular pathways activated, one of the most significant was identified in the activation of BDNF/TrkB signaling downstream of the PI3K/AKT/MAPK pathway, with a neuroprotective role. Another dominant pathway, shared among all examined biofluids, was the one involving WNT/β-catenin and Notch signaling, with a neurodegenerative and reparative role. As expected, inflammatory pathways also figured among the dominant ones, with a significant involvement of IL-2, TGF-β, TLR, and integrin signaling, which were reported to play neuroprotective roles, regulate the pro-inflammatory activity of microglia and astrocytes, and suppress neuro-inflammation and blood–brain barrier disruption following TBI. Center et al. also found that most of the identified pathways were shared among the tested biofluids. Such evidence suggested the activation of a complex interaction between the brain, periphery, and immune system, thus confirming the role of miRNAs in the neuropathophysiology of TBI and their possible value as diagnostic and prognostic biomarkers [5].

2. Traumatic Brain Injury and Exosome-Derived miRNAs

Extracellular vesicles (EVs) are subcellular particles playing an important role in intercellular communication. Their structure consists of a lipid bilayer membrane whose cargo—originated from the parental cell—is variably represented by lipids, proteins, DNA, mRNAs, miRNAs, non-coding RNAs, and organelles. Based on origin and dimensions, EVs can be classified into three main subtypes: exosomes (Exos, 10–100 nm diameter) that originate from the endosomal/multivesicular body (MVB) system and are stored inside the cell before their release; microvesicles (MVs, 100–1000 nm) that originate from a budding process of the plasma membrane; and apoptotic bodies (1–5 nm) that are generated from apoptotic cells and contain degradation products.

All brain cells produce EVs, including neurons, astrocytes, microglia, and oligodendrocytes, but their content is highly variable depending on the external signals. Within a neurological context, they play a key role in modulating synaptic activity and neuronal communication, thus contributing to the pathogenesis of several neurodegenerative processes, including those underlying TBI.

Among EVs, exosomes are of particular interest due to the ability of their miRNA cargo to modulate the gene expression pattern in recipient cells and induce systemic inflammation [7][8][9]. Such a role is highlighted in the work of Long et al. [10], who carried out an experimental study in which TBI brain extracts were used to stimulate the production of exosomes in primary cultured astrocytes; once detected by immunofluorescence, exosomes were separated from astrocytes and added to primary cultured microglia. Subsequent immunofluorescence, qRT-PCR, and western blotting allowed not only confirmation of the exosome uptake by microglia but also the induction of a gene expression pattern consistent with a polarization of microglia into an M2 phenotype with an anti-inflammatory role. A subsequent miRNA microarray analysis of exosomes derived from astrocytes showed a significant up-regulation of 135 different miRNAs, out of which the most represented appeared to be miR-873a-5p, involved in the NF-κB signaling pathway leading to microglial activation. Based on these results, miR-873a-5p expression was evaluated by qRT-PCR in clinical specimens of damaged brain tissue obtained from 15 patients who underwent neurosurgery. Brain tissue samples were all collected three days after the TBI occurrence and consisted of either necrotic brain tissue or severe edema around the necrotic lesion. As a result, miR-873a-5p expression appeared significantly higher within necrotic areas than in the edematous areas. Subsequent in vitro experiments showed that miR-873a-5p suppresses pro-inflammatory factors and promotes the release of anti-inflammatory factors from the microglia by inhibiting both ERK phosphorylation and the NF-κB signaling pathway. Taken together, these findings suggested the role of miR-873a-5p as a possible TBI marker and, simultaneously, as a therapeutic target for improving cerebral injuries and impaired neurological functions.

3. Traumatic Brain Injury and Circulating miRNAs

Compared to the well-known protein TBI biomarkers, circulating miRNAs might be preferable due to their specific characteristics. First of all, their small sizes allow higher stability even in highly degraded samples. Secondly, the high tissue-specific expression gives them a higher sensitivity to the pathology examined. In addition, due to their action at a post-transcriptional level, they can be detected in the early stages of a disease, long before the effects of downstream protein expression are observed. For these reasons, several works have been produced to evaluate the different miRNA profiles in fluid samples of TBI models compared to controls [11].

Time-dependent miRNA expression in cases of severe TBI was evaluated by Ma et al. [12], who analyzed the serum obtained from a total of 20 patients. An RT-PCR analysis was performed, and changes in miRNA profiles were observed at 2, 12, 24, 48, and 72 h, with the following results: miR-18a, miR-203, miR-146a, miR-149, miR-23b, and miR-let-7b showed a >10-fold increase at 12 h compared to the 2 h time-point; all the previously cited except miR-18a showed the same magnitude of increase also at 24 h; miR-181d, miR-29a, and miR-18b showed a >5-fold increase at 48 h; miR-203, miR-146a, and miR-149 showed a >5-fold increase after 72 h. The use of bioinformatic tools helped determine that all the differentially expressed miRNAs were involved in pathways mainly related to cell proliferation, apoptosis, differentiation, inflammatory response, and collagen formation.

Yan et al. [13] evaluated differentially expressed miRNAs between mild, moderate, and severe TBI. An initial array to evaluate the levels of 754 serum miRNAs was performed in two pooled samples of 15 sTBI patients and 15 healthy controls, identifying 19 up-regulated miRNAs in the sTBI group with unfavorable outcomes compared to the

control group. Next, 12 of these 19 miRNAs were selected to be validated by qRT-PCR in the serum samples of a larger cohort consisting of 81 sTBI patients, 81 mTBI patients, and 82 healthy controls. As a result, seven miRNAs (miR-103a-3p, miR-219a-5p, miR-302d-3p, miR-422a, miR-518f-3p, miR-520d-3p, and miR-627) appeared significantly up-regulated in both sTBI and mTBI patients compared to controls, and among these, miR-219a-5p, miR-422a, and miR-520d-3p levels appeared significantly higher in sTBI patients compared to mTBI patients. Yan et al. also investigated the correlation between the expression levels of the seven identified miRNAs with CT lesions; with this aim, 26 TBI patients without head CT and 136 TBI patients with lesions on head CT were analyzed. As a result, miR-103a-3p, miR-219a-5p, miR-302d-3p, miR-422a, and miR-627 levels were significantly higher in TBI patients with lesions than in those without lesions on head CT. Further bioinformatic analyses highlighted the role of up-regulated miR-219a-5p in the inhibition of CCNA2 and CACUL1 expression, thus contributing to the regulation of Akt/Foxo3a and p53/Bcl-2 signaling pathways in neuronal apoptosis activation.

Lastly, Schindler et al. [14] engaged in the evaluation of the levels of six miRNAs (miR-9-5p, miR-124-3p, miR-142-3p, miR-219a-5p, miR-338-3p, and miR-423-3p) in blood samples obtained within six hours after trauma from 33 patients, divided into three groups: severely injured patients without TBI (PT), those with severe TBI (PT + TBI), and patients with isolated TBI (isTBI). The results showed that miR-9-5p, miR-142-3p, and miR-219a-5p could not be detected in any group, while miR-338-3p levels did not show any change between all trauma groups. Interesting results were obtained for miR-423-3p, whose expression significantly increased in patients with severe isTBI, followed by PT + TBI, compared to PT patients without TBI; statistical analyses further showed that miR-423-3p levels positively correlate with TBI severity and risk of mortality, leading to the conclusion that it could represent a promising biomarker to identify severe isolated TBI.

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