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The transcriptome of cerebral ischemia

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Abstract

The molecular causality and response to stroke is complex. Yet, much of the literature examining the molecular response to stroke has focused on targeted pathways that have been well-characterized. Consequently, our understanding of stroke pathophysiology has made little progress by way of clinical therapeutics since tissue plasminogen activator was approved for treatment nearly a decade ago. The lack of clinical translation is in part due to neuron-focused studies, preclinical models of cerebral ischemia and the paradoxical nature of neuro-inflammation. With the evolution of the *Stroke Therapy Academic Industry Roundtable* criteria streamlining research efforts and broad availability of genomic technologies, the ability to decipher the molecular fingerprint of ischemic stroke is on the horizon. This review highlights preclinical microarray findings of the ischemic brain, discusses the transcriptome of cerebral preconditioning and emphasizes the importance of further characterizing the role of the neurovascular unit and peripheral white blood cells in mediating stroke damage and repair within the penumbra.

Keywords

Ischemic stroke; Transcriptome; Microarray; Neurovascular unit; Genomic profiling

1. Introduction

Stroke is broadly defined as a condition caused by occlusion of or hemorrhage from a blood vessel supplying the brain [39]. Clinically, ischemic stroke (IS) is classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) subtype: large-artery atherosclerosis, cardioembolic, small-artery occlusion (lacunar) and undetermined etiology based upon presentation [2,1]. This simple definition does not reflect the heterogeneous clinical

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presentation of stroke (e.g. focal neurological deficits, headaches or loss of consciousness). Despite this heterogeneity, triage of patients in the acute care setting does not significantly differ; treatment frequently occurs prior to determining causality or a definitive diagnosis. As a result, less than 10% of patients receive acute thrombolytic therapy in the emergency setting [80]. These points illustrate the urgent need for a redirection of clinical translational stroke research.

At present, clinical research is focused on selectively studying pathways and choosing drug targets based on prior knowledge, likely obtained from preclinical models. Such a narrow scope may partially explain why promising preclinical studies have not translated into successful clinical treatments. Instead, greater emphasis should be placed on early identifying the molecular etiology and characterizing the body's response to ischemic stroke, specifically pinpointing when injury-to-repair mechanisms occur in the human.

Identifying stroke specific biological markers (biomarkers) is a necessity for successfully determining diagnosis, predicting outcome and delineating therapeutic treatment options [61]. One strategy for establishing biomarkers or pathways that monitor response to therapeutics is global RNA profiling of brain tissue and whole blood. Gene expression profiling simultaneously assesses expression of approximately 23,000 known RNA transcripts without bias of selectively choosing molecular targets. Therefore, transcriptome profiling is ideal for discovering post-stroke molecular targets and/or biological pathways. However, few human studies examine the brain's transcriptome after IS. Complications associated with analyzing human brain tissue include limited accessibility of specimen, decreased molecular integrity of post-mortem tissue, and the limitation of examining end-stage disease processes. Recent studies indicate the peripheral blood transcriptome reflects the cerebral ischemic event. For clinical purposes, blood biomarker studies may give greater insight to the injury and repair process after stroke because blood is readily available in the clinical setting and multiple time points can be assessed. A discussion of human microarray data from peripheral blood post IS will be reviewed, which may be useful for identifying repair processes within the brain and neurovascular unit [6,32,73,78].

This chapter will focus on the cerebral transcriptome of preclinical studies, discuss the transcriptome of whole blood in response to cerebral ischemic injury and emphasize the need for characterizing inflammatory processes of the neurovascular unit following hypoxia.

1.1. Transcriptome of the ischemic brain

Ischemia results in the development of an ischemic core and penumbra. The ischemic core possesses unsalvageable necrotic brain tissue, secondary to lack of cerebral blood flow. The hypoperfused penumbra surrounding the ischemic core is salvageable if blood flow can be restored [25,16], making this tissue an attractive therapeutic target for improving stroke outcome. While restoring blood flow is essential for neuronal survival, reperfusion initiates inflammatory cascades that can contribute to worsening injury. Understanding the beneficial and detrimental effects of neuroinflammation in the penumbra is essential to preserving NVU function and promoting neuron survival post reperfusion.

One of the first studies to investigate the transcriptome of the penumbra after cerebral ischemia examined gene expression changes in the peri-infarction cortex at 2, 4, and 24 h after ischemia [45]. Genes classified as cytokines, chemokines, stress proteins, cell adhesion molecules and immune molecules were extensively regulated in response to cerebral ischemia. Similar findings were obtained from a more recent study of C57BL/J6 mice [66], which demonstrated that at 4 h post-reperfusion, most genes were classified as being related to cell cycle, apoptosis, RNA metabolism or processing, or other cellular and biological processes. At subacute time points (2–4 days) after ischemia, gene expression profiles are associated with transcriptional activation of genes related to delayed neuronal damage and repair mechanisms [5]. These studies reveal a time course of physiological relevant pathways and cell processes involved in the response to cerebral ischemia. Future studies characterizing the regulation of signaling mediators (i.e. kinases, phosphatases, transcription factors and microRNA) in a time-dependant fashion will provide greater insight to the evolution of ischemic injury.

More detailed studies characterizing transcription factors regulation with mRNA expression may reveal a pivotal cross-talk point leading to pathological cell signaling, as mRNA expression varies with time [71]. To predict new transcriptional regulatory mechanisms in the ischemic brain, Ridder et al. applied a computational approach that analyzed microarray expression data and results from a transcription factor promoter binding assay [62]. From this study, two novel transcription factors were identified in relationship to cerebral ischemia: c/ebpβ and vitamin D receptor. These results suggest that computational approaches from microarray data and *in silico* detection of transcription factor binding sites can reliably identify binding sites associated with gene up-regulation. However, no direct relationship between transcription factors and target genes was demonstrated. One possible explanation for this gap between transcription factor and mRNA expression data may lie in the regulation of mRNA, specifically by microRNAs (miRNA).

MicroRNAs (miRNA) are a less understood regulator of mRNA. miRNA are a class of small (18–22 nucleotides) non-coding RNAs that either promote mRNA degradation or enhance translation of target genes [7,42]. Evidence suggests that miRNA producing cofactors and enzymes (i.e. drosha, dicer, pasha, and exportin-5) are not modified post middle cerebral artery occlusion (MCAO) and that miRNA processing plays a significant role in mRNA transcription and translation [13]. miRNA transcriptome reveals that expression similar pathways are altered pathways are similar to those conducted on mRNA transcriptomes. These pathways include inflammation, neuroprotection, receptor function, and ion homeostasis [13]. In developing cortical networks, miRNA expression negatively correlates to the expression of the target mRNA [57]. However, no transcriptome studies have attempted to correlate expression miRNA and mRNA after ischemic stroke.

Specific miRNA changes linked to IS pathology include: astrocyte regulation of water homeostasis (miR-320a) [68], BBB disruption (miR-15a), caspase-induced cell death (miR-497), neuron survival and Hif-1a stabilization (miR-200 family) and proinflamatory (miR-123b) and anti-inflammatory (miR-26a, miR-34a, miR-145) signaling. Moreover, miRNAs mediate processes that coincide with IS risk factors, such as atherosclerosis

(miR-21, miR-126), hyperlipidemia (miR-33, miR125a-5p), hypertension (miR-155), and plaque rupture (miR-22, miR-210) [75,63].

Comparable to mRNA blood studies, brain-specific miRNA has been identified in plasma in both clinical and pre-clinical studies [13,40,31]. Even though preclinical studies have not yielded robust overlap in expression [40], miRNA-210 expression levels correlate with outcome in patients and preclinical models of ischemia [90]. However, clinical application of using miRNA-210 as a biomarker for IS needs to be further studied.

1.2. Effects of ischemic preconditioning on the brain transcriptome

Ischemic preconditioning or ischemic tolerance reprograms the response to ischemia and confers neuroprotection through adaptation to low doses of noxious insults from either peripheral antigen exposure or short-term ischemia [47]. However, preconditioning mechanisms of immune-modulation or mild ischemia are not well-characterized and may differ. Pre-conditioning decreases immune related processes and promotes cell stress responses and detoxification mechanisms; whereas ischemia favors immune and inflammatory mechanisms [81].

Transient MCAO induced several neuroprotective genes and proteins at 3 and 72 h (e.g. heat shock proteins, heme oxygenases, metallothioneins, signal transduction mediators), prevented early gene expression changes and decreased final infarct volume [14]. Similarly, examining the genomic profile of MCAO with and without preconditioning demonstrates expression of similar genes; however, preconditioning results in a substantial down regulation of the common genes expressed [70]. The genomic profile of ischemic tolerance is characterized by dampened expression of genes involved in metabolism, cell cycle regulation, ion channel abundance, controlling excitability, and restricting ATP turnover [81]. These oxygen-influenced signaling mechanisms play a role in the response to preconditioning [64] and endogenous adaptations to oxygen limitation enhance survival in part through protein SUMOylation [29].

The beneficial effects of ischemic preconditioning can also be observed in the regulation of miRNA. miRNAs from the miR-200 and miR-182 families are significantly upregulated 3 h post preconditioning in the ischemic cortex [41], implying selective miRNAs may be involved in neuroprotection. The regulation of miRNAs in physiological and pathophysiological incidences is not well-understood; however, further understanding of miRNA regulation may provide greater insight into mechanisms resulting in brain pathology following IS [30]. Conceptual understanding of preconditioning will give insight to neuroprotection, which may translate to new therapeutic targets [20]. Conversely, many studies investigating preconditioning have not included agedanimals, which may present a variable that alters neuroprotective outcome [17,79].

1.3. Aging and the transcriptome post-stroke

Much of the IS transcriptome data at present has not determined the effect of age on the post-stroke genomic profile. One study highlighted the importance of age post IS and confirmed a difference in gene expression in the ipsilateral and contralateral cortex in young and old rats following transient ischemia [9]. Specifically, the peri-infarct region of aged rats

showed early upregulation of genes associated with DNA damage and down-regulation of anti-apoptosis related genes [9]. Aged rats also demonstrated impaired neurogenesis in peri-infarct regions and markedly increased expression of inflammatory and glial scar-forming genes. Both findings indicate a propensity towards greater tissue damage with age, which may explain the worsened stroke outcome observed in older patients. Interestingly, impaired neurogenesis and increased BBB permeability has been observed in the contralateral hemisphere for both aged and young rats [9,36]. The alterations observed in the contralateral hemisphere may influence mechanisms influencing stroke outcome such as neurogenesis and neuroplasticity. A better understanding of the molecular mechanisms occurring in the contralateral hemisphere is needed, particularly in aged animals.

2. Aging of the neurovascular unit

The NVU is a complex intracellular network involving the cerebral capillary endothelial cells, astrocytes, pericytes, and microglia, which work together to establish, maintain, and regulate neuronal microenvironments. Aging affects morphology and function of NVU cells. The cerebral endothelium expresses less elastin and collagen [58] and undergoes vascular thickening [4], thereby impairing cerebral autoregulation. Additionally, the neuron's ability to propagate neurotransmission [36] and secrete neurotransmitters is altered [8,67]. Age-associated dysregulation of NVU signaling may be the initial step in neuroinflammation leading to altered homeostasis within the NVU. As a result, aged microglia appear to mediate neuron death instead of neuroprotection [86]. A recent study examining the sprouting transcriptome post stroke identified that both age and time post IS affect growth factors, cell adhesion, axonal guidance, and cytoskeleton expression [43]. Signaling of repair and regeneration cues appear to be altered by the pro-inflammatory environment of the aged-brain [86]. The complexities of neuroinflammation within the NVU are further compounded by stroke risk factors such as diabetes [82], dyslipidemia [69] hypertension [83] and pro-oxidant cell signaling, all of which may exacerbate insult and modify immune-related mechanisms. It is no surprise that aged animals exhibit worsened stroke outcomes [51,3,37,65], increased infarct volume, higher mortality [83,51] and altered response to therapeutics [36], when compared to young animals after cerebral ischemia. Similarly, it has been estimated that each year of age results in approximately one percent more penumbral tissue post IS [4]. Despite this known discrepancy between age and favorable outcome, many studies examining ischemia and post-stroke neuroinflammation have been conducted using young animals. An urgent need exists for more studies regarding the genomic response of the ischemic aged brain. In this section, neuroinflammatory signaling mechanisms affecting the different cellular components of the NVU will be examined. Pericytes have been omitted from the discussion due to a lack of genomic related studies, however investigations of pericyte function following hypoxia are warranted.

2.1. Gene expression of the neurovascular unit post ischemic stroke

In vitro models are useful for elucidating mechanisms of hypoxia on specific NVU cell types or modeling cell–cell interactions using co-culture models. However, *in vitro* methods lack the complexity or heterogeneity of signaling at the tissue level. Methods for analyzing the "at risk" tissue after experimental IS are beneficial for identifying region-specific

signaling leading to neurodegeneration or neuron survival [92,26]. An elegantly designed study by Li et al. used microdissection to selectively isolate neurons from penumbra tissue to examine transcriptomes. Interestingly, this study highlighted a paradoxical up-regulation of myelin and ephrin receptors sprouting in neurons of aged mice brains [43]. Other evidence suggests that region specific responses to ischemia and post ischemic mechanisms of damage may differ, indicating that regional response to therapeutics may differ as well [52]. Transcriptome analysis is needed to identify NVU cell-specific responses to hypoxia and elucidate cross-talk mechanisms between NVU specific and regional studies, thereby broadening the knowledge base for treatment of IS and possibly other neurodegenerative diseases. In this section, neuroinflammatory signaling mechanisms affecting the different cellular components of the NVU will be examined.

2.2. Blood–brain barrier

Co-morbid diseases are a primary cause initiating microvascular dysfunction. Given the highly specialized function of the BBB, understanding pathophysiological genomics may improve our understanding of normal and disease biology. BBB genomics is the study of brain capillary-derived RNA and involves isolation of brain capillaries [59]. A study examining BBB genomics has been performed on cerebral capillaries of stroke-prone spontaneously hypertensive rats (SHRSP) and stroke-resistant spontaneously hypertensive rats using suppression subtractive hybridization in combination with a cDNA filter screening step [38]. This study revealed many known and unknown genes with altered expression in SHRSP. Of particular interest is a G protein related marker, RGS5, which was markedly decreased in cerebral capillaries of SHRSP. These findings suggest G protein signaling may be a relevant biomarker for hypertension induced vascular disease and IS. Another study examining the BBB response to global ischemia at 1, 6 and 24 post ischemia revealed differential protein expression between early (1 h) and late stages (6 and 24 h) following reperfusion. Early reperfusion is associated with patterns of decreased ion pumps, nutrient transporters and cell structure proteins with an increased expression of transcription factors and signaling mediators. Increased signs of inflammation and microvascular remodeling were expressed in late reperfusion [23]. An interesting avenue for future investigations would be to examine the effect of ischemia reperfusion on animal models with stroke risk factors (i.e. OB/OB obese mice or SHRSP).

2.3. Astrocytes

Astrocytes are specialized glia cells that serve many functions: regulating metabolic factors (e.g. glucose, neurotransmitters, ions, blood flow, etc.) [55], altering BBB integrity through factor secretion at the endfeet [27,15], and modifying brain water volume through aquaporin channels [87]. Hypoxia influences aquaporin isoform expression [88]. Silencing of aquaporin 4 (AQP4) coincides with decreased expression of ischemia-related genes [56], suggesting that AQP4 may be a major player in cerebral edema post-stroke. Other astrocyte specific responses to hypoxia include upregulation of glycolytic and angiogenic factors [50], which differs from the cerebral endothelial hypoxic response. Gap and adherens junctions at the astrocytic end feet provide a mode of intercellular communication with the endothelium and surrounding cells. When exposed to CNS pathology like IS or traumatic brain injury (TBI), astrocytes become activated and rapidly express connexon-43 [24]. Reactive

astrocytes surrounding the penumbral region after TBI selectively up-regulate connexon isoforms-20 and -43 [53] and may play an important role in the degree of astrocyte reactivity. miRNA profiling of primary astrocyte cultures reveals that reactivity is linked to altered miRNA regulation of the TNF-α pathway [54]. Reactive astrocytes display a genomic profile both *in vivo* and *in vitro* that is distinct from hyperproliferating (scar forming) or neoplastic (tumorogenic) glia [10]. Moreover, a study using bioinformatic approaches to compare microarray data from multiple studies identified a set of astrocyte-specific genes, which varied regionally within the CNS [5]. These findings suggest that subpopulations of astrocytes may differ in physiological roles, much like neurons. Further study of astrocyte subpopulations following brain injury and disease may lead to a better understanding of astrocyte function in the NVU.

2.4. Microglia

Microglia, stemming from the monocyte lineage, generate an innate immune response within the CNS when the brain is exposed to injury, ischemia, or an inflammatory stimulus. Transformation from stellate to a ramified shape permits the microglia to initiate an inflammatory response within the brain through cell proliferation, moving to the site of injury, and engulfing cell debris [72]. Evidence from primary microglia gene expression analysis suggests Wnt signaling may play a role in promoting the physical change of microglia post ischemia and correlates with pro-inflammatory microglia signaling [22]. In the aged brain, increased expression of transforming growth factor B appears to play a primary role in the pro- inflammatory cascade of glial signaling post IS [18]. Over activation of microglia may lead to aberrant JAK/STAT and/or cyclo-oxygenase signaling. Excessive pro-inflammatory signaling promotes lipid oxidation and potentially increased damage due to the lipid-rich nature of the brain. Thus, it is plausible that activated microglia act much like transformed macrophages or foam cells in the periphery, promoting pathology and exacerbating damage.

2.5. Neuron fate – apoptosis necrosis or survival?

Neurons lack a metabolic reserve and require a constant supply of oxygen and metabolites (e.g. glucose) to function. As a result, the neuron is extremely sensitive to changes within the NVU-established microenvironment (e.g. ion fluxes, osmotic alterations, pH, etc.). *In vitro*, the neuron response to hypoxia indicates up-regulated cytoskeleton-related proteins (i.e. fibronectin, integrin a, doublecortin kinase), indicating cellular stress responses [34]. Expression data further supports stress-signaling cascades by upregulating endoplasmic reticulum proteins and ubiquitin mediators [51]. Neuron stress results from detrimental changes in the microenvironment, including insufficient metabolic supplies, excess extracellular glutamate, calcium overload, acidosis and oxidative damage. The extent of exposure to such factors will ultimately influence the fate of the neuron. This section reviews signaling cascades at the acute (minutes to a few hours), subacute (few hours to a few days), and chronic phases (may last a few months post-stroke) of signaling that influence neuronal fate.

When vessel occlusion occurs, energy failure promotes necrotic neuron death at the ischemic core. Cell debris released from necrotic tissue contributes to stress signaling

cascades affecting the penumbral tissue. Other factors influencing penumbral viability include decreased cerebral blood flow which alters energy-dependent regulation of ion homeostasis and subsequently brain volume [35]. Altered ion homeostasis promotes increased intracellular calcium thereby promoting glutamate release. Excitoxicity and cortical depression contribute to the expanding infarct area. During the acute phase, reperfusion initiates increased pro-oxidant stress from mitochondrial dysfunction [44] and pro-apoptotic signaling from toll-like receptor isoforms [79]. While reinstatement of blood flow is essential to save neurons, reperfusion increases neuronal injury by increasing reactive oxygen species (ROS) and enhancing the inflammatory response. Leading into the subacute phase and chronic phases, a delicate balance between inflammation and ROS must exist. Some inflammation and ROS are required to initiate repair. However, over expression of inflammatory mediators and excessive ROS exacerbate damage. The dual nature of the inflammatory response may explain why anti-inflammatory drugs have not succeeded in clinical trials [91,28].

The primary mechanism dictating neuron survival stems from mitochondrial function and signaling. Oxygen–glucose deprivation and oxidative damage to mitochondria result in decreased ATP production, which contribute to initiation of death signaling cascades. Mitochondrial signaling cascades regulating delayed ischemic apoptosis are up regulated in the early and late phases of ischemia [49]. Mitochondria play a key role in regulating caspase-mediated apoptosis (e.g. Casp 3 or Casp 8) or survival signaling involving the Bcl family (e.g. Bcl-2, Bax, and Bim). *In vitro* evidence suggests that blocking caspase activity or enhancing Bcl-2 expression promotes survival after ischemic insult [49].

3. Translating findings from the bench to the bedside

Translational research promotes the concept of using findings at the bench in efforts to improve patient care. Perhaps, reversing this process may further clinical application by using humans to identify biomarkers and using animal models to study gene functionality. One study of interest used a translational approach by comparing the IS transcriptome of the human brain to the transcriptome of MCAO rats at various time points [52]. These findings confirmed previously reported genes involved in IS, such as those participating in transcription, apoptosis, inflammation and neuroprotection. However, there were significant differences between gene expression patterns in humans and rats, and few studies have examined the human brain transcriptome post IS.

Animal models of MCAO may not entirely reflect the pathophysiological process of human stroke. While species diversity is one proposed reason for the current lack of success at bedside translation, comparative genomics may help to eliminate this disparity. As more human gene expression studies are published, comparisons among clinical and preclinical studies will be essential to determine the relevance of proposed markers and pathways [21]. Additionally, the Stroke Therapy Academic Industry Roundtable (STAIR) criteria [19] reinforce the need for defining histological and functional outcomes in multiple animal species, as well as, monitoring time windows and dose response for potential therapies. Biological activity of a therapy remains a challenge in translating preclinical data to humans.

Overall, there is a lack of robust or operational biomarkers to substitute for clinical endpoints for IS. Currently, blood remains the most readily accessible tissue in the clinic. If blood based biomarkers for IS can be identified, then gene functionality can be studied using *in vivo* and *in vitro* methods.

3.1. Peripheral white blood cells mirror the genomic profile of CNS injury

Tang and colleagues studied gene expression patterns in rats subjected to IS, intracerebral hemorrhage, status epilepticus, and insulin-induced hypoglycemia [76,77]. They identified gene expression profiles in the peripheral blood for each of the injuries 24 h after the insult, providing proof of the concept that gene expression profiles of the peripheral blood accurately reflect acute brain injury. Moreover, bone marrow derived stromal stem cells (BMSC) infiltrating the post-stroke brain show persistent epigenetic gene expression that differs from naïve BMSC [89]. These expression changes favor neuroprotection, regeneration and angiogenesis.

Specific populations of immune cells and gene expression profiles in the peripheral blood may reflect incidence of neurological disease, including IS [78]. Two studies examining the transcriptome of whole blood post stroke have identified an overlapping mRNA profile for IS [6,78]. Among the similar genes expressed, up regulation of MMP-9 has been linked to increased enzyme activity in the peripheral blood and worsened BBB permeability following stroke [36]. Understanding gene expression changes in the peripheral blood may help guide the study of molecular mechanisms contributing to cerebral damage in the brain. A clinical study suggests miRNA profiling lead to a suitable biomarker for IS diagnosis and prediction for prognosis [73,74]. Although preclinical studies have not yielded a robust overlap in miRNA expression with the clinical study [14], miRNA-210 shows promise as a biomarker for stroke. A translational study by Zheng et al. identified miRNA-210 in the blood of patients with IS and mice exposed to cerebral ischemia. miRNA-210 expression positively correlated with better outcome in an IS patient population, suggesting an miRNA biomarker for stroke [90]. The role of specific miRNAs in stroke etiology and pathology is an important area that warrants further investigation.

Genomic profiles may also be useful for identifying stroke subtype or monitoring response to treatments. For example, mRNA expression profiles can differentiate cardio-embolic from large vessel IS [32], where as miRNA expression profiles correlate with acute stroke treatment classification [73]. With regard to monitoring response to treatment, one study in an ischemic rat model showed that tPA reperfusion influenced gene expression independent of ischemia and that the identified genes were related to immunomodulation [33].

Interestingly, analysis of the human middle cerebral artery post-thromboembolic stroke yielded similar results [85]. Major pathways found to be activated were immune response mediators, signal transduction factors, transcriptional activators, and metabolism related genes, which are similar to that which has been identified in preclinical models. Carotid plaque from symptomatic patients demonstrates a similar immune profile showing changes of neoplastic-like growth mediators, angiogenic factors, signal transduction intermediates and metabolism mediators with a predisposition toward neurodegenerative diseases [84].

These findings suggest that a complex interrelationship exists between immune, vascular, and brain function.

3.2. Benefits and limitations of microarray analysis in stroke research

Computational approaches to large microarray data sets must include tests of threshold significance to safeguard against false discovery. Moreover, emerging databases that link to Pubmed or the National Center for Biotechnology Institute (NCBI) allow an investigator to compare one's data with other studies. A review by Van Elzen et al. evaluated expression profiling data from *in vivo* and *in vitro* studies investigating the response to ischemia or hypoxia and demonstrated that gene expression differs by stroke model, but gene subsets dictating biological processes and pathways are similar [81].

There is a large knowledge gap regarding protein expression and post-translational regulation following IS, as only a limited number of studies have been published. Ischemia suppresses protein synthesis and alters pathway signaling through post-translational modification (e.g. sumoylation, phosphorylation, glycosylation). Ubiquitination and sumoylation are protein modifications that greatly impact protein turn-over and are associated with transient ischemic stress [93]. Yet, the post-translational signaling cascades are not well-characterized post-IS. Moreover, translation state analysis has shown that many transcripts induced following cerebral ischemia are not efficiently translated [46]. Few proteins associated with cell death have been identified. However, there does appear to be effective translation of heat-shock and antioxidant proteins, anti-inflammatory agents, and kinase signaling systems. Questions regarding the benefit of accumulating data on transcripts that fail to be translated are of concern [46], which shows the need for comprehensive data sets of protein expression in the ischemic brain.

The missing link to a better understanding of the genomics and proteomics of IS may lie in the regulation of ribosomal proteins. Ribosomal proteins facilitate translation of messenger RNA (mRNA) to protein and may be sequestered into non-functional protein aggregates following reperfusion [12]. Ubiquitin co-localizes with ribosome subunits and the mRNA regulatory protein T-cell internal antigen 1 (TIA-1). Involvement of TIA-1 with post-ischemic protein aggregates suggests that neurons possess the ability to present or process antigens [12]. This may serve as a signaling mechanism for propagating the transcriptome of cerebral injury to peripheral immune cells. While it is known that peripheral immune cells enhance inflammatory signaling in the brain [48], mechanisms guiding such processes are largely unknown. Future transcriptome studies of both brain and blood may reveal mechanisms of cross-talk between the central and peripheral immune systems.

4. Conclusion

Over 20 years ago, David O. Wiebers wrote an editorial in Stroke that predicted issues with regard to our research efforts, "An over-reliance on [animal] models may impede rather than advance scientific progress in the treatment of [stroke]. The complexities of creating a truly representative model for human IS go far beyond developing ways to occlude a cerebral artery in a given animal [66]." To move forward, emphasis must be placed on setting strict criteria for the preclinical evaluation of neurotherapeutics and diagnostics, as recommended

by the STAIR criteria [19]. Similar guidelines should be directed toward efforts characterizing the paradoxical nature of neuroinflammation by examining the effect of ischemia at different time points post-injury, assessing the effect of ischemia on all cell types of the NVU while taking brain region variability into consideration. Inflammatory and immune responses of supporting NVU cells (astrocyte, microglia, pericyte, cerebral endothelium) are equally important in determining neuron viability and the microenvironment for neuron subpopulations may vary. The reproducibility of IS models can be used to decipher complex regional differences to ischemia, which is often presented in the clinical population.

Moving forward, clinical stroke populations and genomic technologies are an essential starting point for identifying IS biomarkers and/or potential therapeutics. The heterogenous nature of stroke will likely lead to a panel of IS specific biomarkers for clinical diagnosis and/or multiple therapeutics for successful treatment. Through this process, preclinical studies will serve as means to characterize molecular and physiological functionality of biomarkers and to screen potential therapeutics in aged models of ischemia. If IS biomarker development follows the Biomarker Task Force published guidelines [11] and potential drugs or medical devices are tested on evolutionarily advanced species beyond the rodent [19], then advancements in the diagnosis and treatment of IS are on the horizon.

Abbreviations

IS	ischemic stroke
TOAST	Acute Stroke Treatment subtype
biomarkers	biological markers
NVU	neurovascular unit
BBB	blood–brain barrier
mRNA	messenger RNA (mRNA)
c/ebpβ	Ccaat-enhancer-binding protein
miRNA	microRNA
MCAO	middle cerebral artery occlusion
SHRSP	stroke-prone spontaneously hypertensive rats (SHRSP)
AQP4	aquaporin-4 (AQP4)
TBI	traumatic brain injury
TNF-α	tissue necrosis factor alpha
ROS	reactive oxygen species
STAIR	Stroke Therapy Academic Industry Roundtable
BMSC	bone marrow derived stromal stem cells
NCBI	National Center for Biotechnology Institute

TIA-1 T-cell internal antigen 1

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