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Prospects of Single Cell Omics (SCO) Analysis for Investigating Nervous System Disorders

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Abstract

Single Cell Omics (SCO) is an evolving field in biomedical research which offers prime application in neurosciences. The human nervous system is complex and shows variability in cell types. It includes neurons (functional components), glial cells (supporting cells), astrocytes (provide nourishment), oligodendrocytes (synthesize myelin sheath), and microglia (defense mechanism). To understand the functional and disease states of the nervous system, it is essential to investigate them at the single-cell level. It has been estimated that every 1 in 9 people is affected by mental or neurological disorders, including psychological disorders (generally referred to as mental disorders), psychotic disorders (involving psychosis), and neurological disorders (involving neurological factors), globally. These disorders have multifactorial etiology and are caused by genetic and environmental factors. Every disorder has distinct pathophysiology affecting multiple brain regions. SCO has excellent potential to provide insight regarding the diagnosis, pathophysiology, and treatment of neurological disorders. The stringent well directed SCO methods enhance the understanding of complex nervous system disorders, such as meningitis, stroke, schizophrenia, Parkinson's disease, and Alzheimer's disease, which paves the way for future research.

Keywords: Alzheimer's disease, genomics, metabolomics, Parkinson's disease, proteomics, Single Cell Omics (SCO), schizophrenia, transcriptomics

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Introduction

Single Cell Omics (SCO) is a new field in biology in which genome wide data is extracted at the level of individual cell. This technology has a broad scope and it covers fundamental to high throughput techniques, such as imaging, mass spectrometry, and DNA RNA sequencing. It is further supported by a powerful software which provides diverse interpretations regarding cellular differentiation, development, and regulation. The understanding of complex diseases such as cancer, which involves heterogeneous cell populations, has been improved via SCO. This technology is still in its infancy, although it remains on track for progress at single cell level [1].

Human brain is the control center of the body. It is a complex organ, consisting of numerous neurons and synaptic connections which form the neuroanatomical subdivisions of the adult brain [2]. The brain also consists of neuroglia (glial cells), supporting cells which synthesize important regions [3]. It contains a clear liquid which is present within and around the different organs of the Central Nervous System (CNS). This liquid is known as Cerebrospinal Fluid (CSF). It provides support, acts as a shock absorber, and clears wastes [4].

There are three types of glial cells known as astrocytes, microglia, and oligodendrocytes, respectively. Oligodendrocytes are responsible for membrane synthesis which insulates axons through a multilayer myelin sheath [5]. Astrocytes play a vital role in the maintenance of extracellular ion balance, recycling neurotransmitters, shaping synaptic circuits, and regulating the blood brain barrier which prevents the entry of pathogens into the brain [6]. Microglial cells provide immunity and maintain brain homeostasis [7].

Evolution has reshaped the mammalian brain; it has transformed into a complex organ consisting of a variety of cells [8]. The brain cannot be adequately analyzed on the basis of bulk cells since the latter are hugely diverse. These cells are responsible to make complex cortical circuits. However, advancements in SCO have provided high resolution for single cell analysis [9].

Genomics of the Nervous System

Single cell genomics provides the understanding of cell-to-cell genetic

diversity that occurs due to intracellular variation of the genome, epigenome, proteome, and metabolome. The study of genomics and related disciplines has progressed rapidly over the years, opening new doors for the analysis of genome organization and behavior at single cell level. It also allows the investigation of molecular signatures with high resolution. The analysis of genome at sequence level provides gene expression profiles of individual cells [10, 11].

Single cell genomics constitutes a valuable resource for analyzing the human brain [12, 13]. Diversity in neuron occurs through somatic variations of genome and epigenome. It relates to aneuploidy, chromosomal instability, and intercellular changes in epigenetic profiling [14, 15]. Chromosomal instability, also knowns as aneuploidy, is likely to play its role in brain aging [16, 17]. Somatic genome variations may be associated with non-malignant brain diseases which affect the brain tissue [18–20].

Somatic genome variations increase the susceptibility towards non-malignant brain diseases which affect the brain tissue [12, 13, 16]. A variety of nervous system disorders including neuropsychiatric and neurodegenerative disorders occur due to the changes in gene expression profiles mediated by intracellular epigenome variations [21]. The data generated from brain cell population at different levels of genome, epigenome, proteome, and metabolome provides separate paths. This induces the lack of a unified approach regarding systems biology that explains human neuronal diversity and the pathophysiology of nervous disorders. Indeed, biomedicine has important applications for genetic and epigenetic profiling at different levels of genome organization [12, 13, 15].

Transcriptomics of the Nervous System

Transcriptome is a critical component of the living system. It is dynamic in nature and provides essential information about cell type and cell state. Distinct categories of cells express specific markers which differentiate them from other cell types [22, 23]. Transcriptome has a broader scope and coverage at the single cell level while performing technical and experimental techniques. This is confirmed by the fact that transcriptome has higher copy numbers per cell as compared to the genome. It ranges from

1 to 100000 per cell and generally, at least 100 copies per cell are present [24].

Human brain, being a complex organ, depicts great utility for transcriptomic analysis comprising different cell types from neurons to oligodendrocytes [25–27]. Transcriptomic techniques have certain limitations when the whole brain structure or its substructures are analyzed. The bulk tissue does not provide precise and stringent results that may occur at the single cell level [28, 29].

Proteomics of the Nervous System

Neuro-proteomics is a subfield of proteomics and relates to the proteins of cells, tissues, and organs which make up the nervous system [30, 31]. It encompasses the identification of proteins, as well as investigating the role of post-translational modifications and changes in the proteome which occur due to age, genetic, and environmental factors. These factors are also responsible for the onset of disease conditions [32, 33].

The proteome of brain is complex. It is divided into various sub-regions where every region performs a specialized function. For example, cerebral cortex and its associated regions are responsible for sensory perception and decision-making. Hippocampus is involved in spatial learning, whereas striatum coordinates movements and reward-based learning. Amygdala controls fear and emotional responses [2]. Central Nervous System (CNS) is a very important resource for neuronal proteins and 30%-50% of the mammalian genes are expressed in this area. Differential expression of genes can allow us to understand the complexity of CNS [31]. Proteomics allows us to trace the function of genes and is aligned with functional genomics [34].

Traditionally, protein samples were analyzed using two-dimensional (2D) gels, where multiple proteins were displayed and analyzed via a single gel [35, 36]. The interpretation of 2D gel data was difficult and applicable only for abundantly found proteins. This problem was solved by Mass Spectrometry (MS). It provides a diverse analysis of proteins present in a single sample based on different parameters, such as protein identification, determination of molecular weight, and characterization of post-translational modifications [37]. MS is a reliable, efficient, and diversified technique, especially with some modifications such as Matrix Assisted Laser Desorption/Ionization (MALDI) and Electrospray Ionization (ESI).

In MALDI, a laser is used to ionize the protein sample before it is analyzed, whereas in ESI a strong electric field is used to ionize the sample before investigation [38].

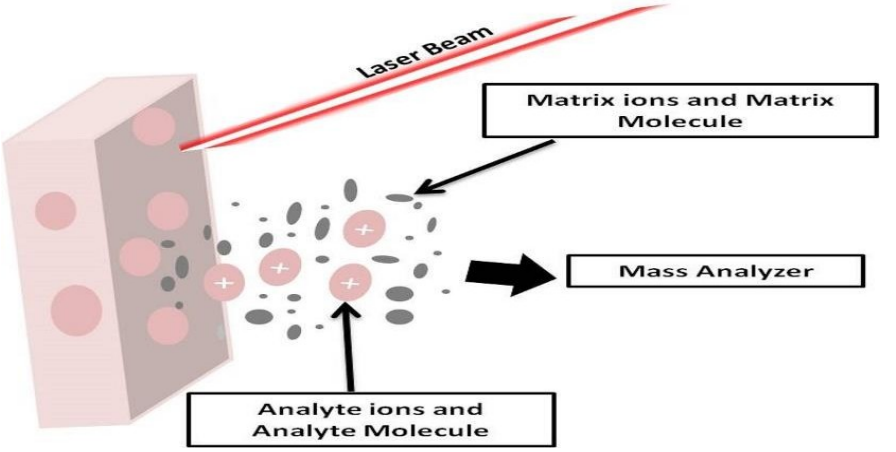

Figure 1. A schematic diagram of MALDI indicating the matrix containing analyte, that is, the protein to be analyzed. When laser beam is bombarded on the matrix, the analyte and matrix molecules are ionized. These ionized molecules are further analyzed using Mass Analyzer.

Metabolomics of the Nervous System

Metabolome is a critical component in terms of cellular phenotype [39]. It has important applications in general screening and identifies phenotypic differences in a precise manner. It is a powerful technique for making population level measurements. However, since cell populations are not homogeneous, the study at single cell level becomes critical to perform metabolomic analysis. Cell populations vary in microenvironment, developmental stages, and according to the age of the cells [40].

Brain is metabolically active and accounts for 20% of the total energy consumption in the body of human beings [41]. Energy requirements need to be addressed to ensure signaling and cognitive activity in the nervous system. Evolution has increased the expression of genes associated with energy production in human cortex in comparison to other non-human primates, which highlights the importance of brain energy metabolism [42, 43].

Single Cell Omics (Sco) In Nervous System Disorders

Scientists are currently investigating nervous system disorders due to their high prevalence, worldwide (one out of every nine individuals dies due to these disorders) [44]. Variations in the clinical implications of these disorders depend on the site where malfunctioning occurs in the nervous system. CNS consists of distinct cell types expressed differently [45]. Hence, techniques based on the analysis of these distinct cell types have a high potential to effectively diagnose nervous system disorders.

Meningitis

Infectious agents, such as bacteria, viruses, and fungi, can affect the nervous system and may lead to various clinical conditions. In order to understand the effect of these agents on the nervous system, let's take the example of meningitis. It is a bacterial infection of the nervous system characterized by the inflammation of meninges, where the meninges layers of arachnoid and pia mater are affected the most [46]. This infection can affect brain parenchyma [47], ventricles, and spinal cord [48].

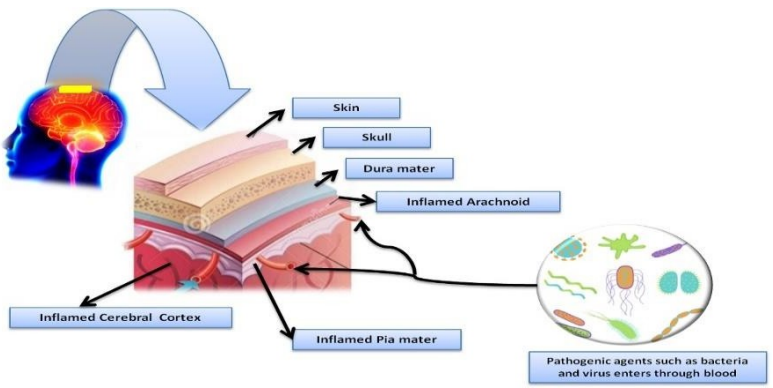

Figure 2. Meningeal inflammations of the brain

A segment of the brain is shown with different layers of skin, skull, and meninges (pia mater, dura mater, and arachnoid). The meninges layers of pia mater and arachnoid are affected by pathogens, such as bacteria. Cerebral cortex may also get affected by meninges inflammation.

Other factors, such as viruses, fungi, neoplastic diseases, and drug reactions can also lead to meningitis. Bacterial species causing meningitis include hemophilus influenza, pneumococci, and others. The risk of

hemophilus infection in human beings has been minimized through vaccination but pneumococci still poses the threat of inducing meningitis to children and adults. Its incidence is low in the United States (US) [49, 50] and Western Europe [51] as compared to Africa [52]. Although, the use of conjugate pneumococcal vaccines has reduced meningitis [53], still resistance against the beta-lactam antibiotic has increased its prevalence [54].

Stroke

Vascular dysfunction has always posed a serious threat to humanity and remains a significant cause of death, globally. Nervous system is also affected by vascular dysfunction, as in the case of stroke. It is a type of cardiovascular disease (CVD) which occurs mostly due to ischemia (restriction of blood supply to tissues, especially brain tissues) [55]. In some cases, it occurs due to cerebral hemorrhage caused by the bursting of brain artery [56].

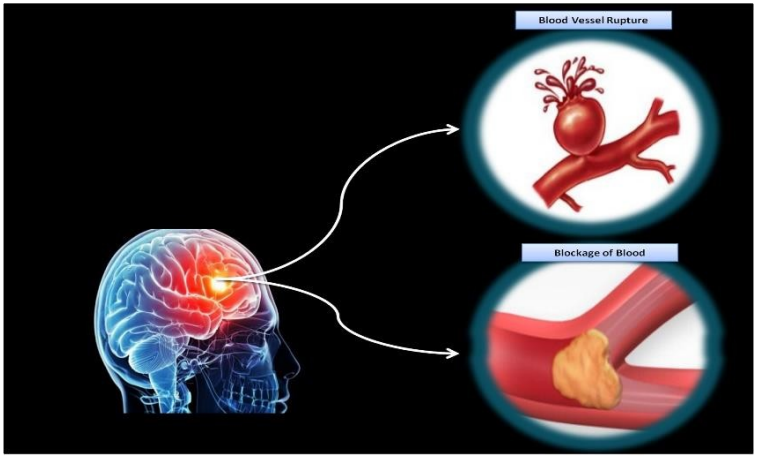

Figure 3. Pathological mechanisms of stroke: hemorrhage (bursting of the brain artery) and ischemia (blockage in the supply of blood).

The brain gets nutrients and oxygen from the blood, therefore, a stroke can be fatal [57]. Even a brief interruption of blood supply to the brain can cause permanent damage to it. The management of stroke involves improvement in blood supply and protection of brain tissue against ischemia [58].

Parkinson's disease

A very important function of the nervous system is to coordinate motor functions. These functions are essential for locomotion and provide animals with the ability to move from one place to another. Motor function is affected when the brain regions are damaged due to different neurodegenerative diseases including Parkinson's disease [59].

Parkinson's disease is a motor dysfunction of old age in which neurodegeneration of different brain parts can lead to clinical implications of postural instability, resting tremor, and bradykinesia. In this disorder, dopaminergic neurons are significantly reduced in the substantial nigra (SN) pars compacta region, while nigral neurodegeneration in the presence of intracytoplasmic inclusions (Lewy Bodies) of the surviving neurons remain the pathological hallmark of Parkinson's disease and make diagnosis effective at the time of autopsy [60, 61]. Dopamine replacement therapy is used to minimize the symptoms of Parkinson's disease; still, the disease remains incurable as the loss of dopaminergic neurons cannot be reversed [62].

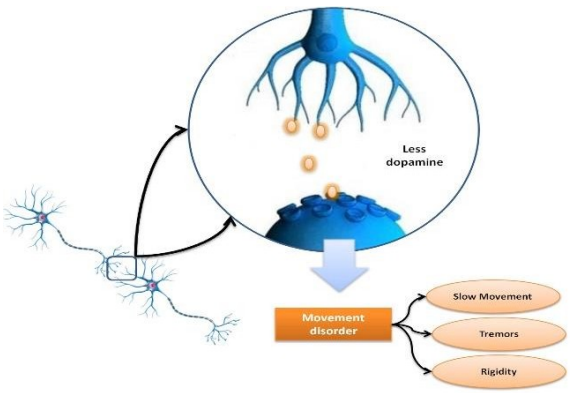

Figure 4. Pre- and post-dopaminergic neuron in Parkinson's disease

The hallmark of this disease is the reduced quantity of dopaminergic neurons which leads to lower dopamine levels and clinical implications, such as slow movement, tremors, and rigidity.

Schizophrenia

Cognition, another important function of the brain, is the source of decision-making, thought, and reasoning. A person suffering from cognitive

problems is unable to make decisions, give logic, and express thoughts. Schizophrenia, for example, is a severe neuropsychiatric disorder which affects 1% of the world's population and is characterized by strong environmental, genomic, and neurodevelopmental associations [63]. The patients diagnosed with schizophrenia are at a greater risk of mortality and morbidity. They are also more prone to substance abuse and suicide [64, 65]. Schizophrenia mainly involves two types of symptoms, labeled as positive and negative symptoms, respectively. Positive symptoms include catatonic (abnormal) behavior, hallucinations, disorganized speech, and delusions, whereas negative symptoms include emotional weakness, neglect, and avolition (lack of motivation) [66].

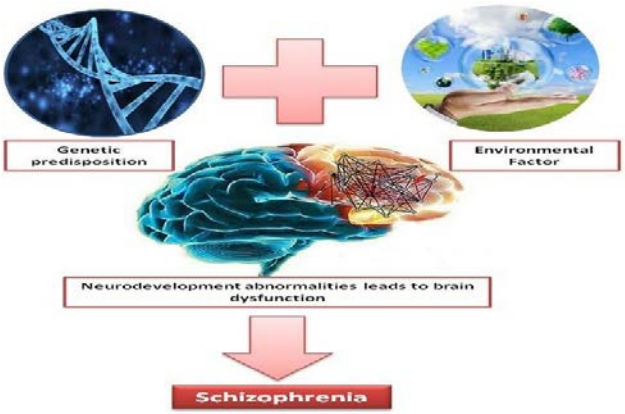

Figure: 5 Factors responsible for the pathophysiology of schizophrenia

The onset of disease is in adolescence but symptoms become severe at the later stages of life [67]. Treatment is mainly carried out through pharmacological intervention using antipsychotic drugs that block dopamine receptors, although non-pharmacological methods, such as psychotherapy, are also performed [68, 69].

Alzheimer's Disease

Alzheimer's disease (AD) is a multifactorial genetic disease with the distinguishing feature of neurodegenerative dementia. There is no treatment for AD and its high prevalence causes serious threat to the health care system. The patients experience deficiency in cognitive capabilities, initial loss of episodic memory leading to dependency, and ultimately death [70].

The brain of an AD patient experiences progressive and cortical degeneration which can be visualized through neuroimaging and macroscopic examination. The examination revealed the presence of Neurofibrillary Tangles (NFTs) of hyperphosphorylated tau protein and extracellular accumulation of amyloid-β (Aβ)1–42 peptide [71].

In AD, transcriptome studies make possible the investigation of transcriptional regulation in the brain. Transcriptomic analysis is mostly conducted through postmortem material and storage conditions are critical for transcriptome stability and preservation. The difference between diseased and healthy tissues can be identified through the differential expression of genes responsible for synapse function in neurons, as well as through inflammatory response genes in the case of microglia. Reactive gliosis can provide a clear contrast between healthy and affected tissues [72].

The mouse models used to study early and late changes in gene expression provide key insights useful to understand the pathophysiology of AD. Mouse models harboring the human Amyloid Precursor Protein (APP), Microtubule associated tau protein (MAPT), and Presenilin1 (PSEN1) mutations explain the tau or amyloid pathology demonstrated in AD [73].

Significance of SCO in Nervous System Disorders

SCO is a technology that brings both challenges and opportunities. A large amount of data is currently generated by SCO, when this data is directed for analysis its cost is raised. To reduce the economic cost, an integrative approach needs to be developed. There is a need to integrate bioinformatics with SCO, so that effective statistical analysis can be performed and automated stringent output can be generated [74]. Secondly, it is a good practice to perform live cell imaging rather than lysing the cell to study its constituents. The results from these experiments can be investigated through *in situ* techniques. This would pave the way for biomarker identification and pathway determination [75].

SCO technology is rapidly advancing as it is directed towards the analysis of metagenomes, pathway mapping, and sophisticated protein-protein interaction. This advancement in high throughput techniques used in genome wide association studies remains productive and leads to efficient results that can be interpreted and analyzed easily. The discovery

of these critical mechanisms and pathways improves the prevention and treatment of disorders. Biological processes can be targeted for testing new drugs. The complexity of disorders would be minimized by SCO and it would provide a path to elucidate biological processes [10].

Conclusion

SCO is a rapidly evolving technology which aims to develop diverse methods and techniques to resolve biological problems. This technology has a broad scope which includes genomics, transcriptomics, proteomics, and metabolomics. It enables the researchers to keenly monitor and analyze biological samples at single cell level. We know that nervous system is a complex entity composed of a variety of cells. SCO technology provides a technical means to analyze the nervous system and improve our understanding regarding its structure, function, and disorders.

List of Abbreviations

SCO Single Cell Omics

CNS Central Nervous System

2D Two Dimensional

MS Mass Spectrometry

MALDI Matrix Assisted Laser Desorption/Ionization

ESI Electrospray Ionization

US United States

CVD Cardiovascular Disease

SN Substantia nigra

AD Alzheimer disease

NFTs Neurofibrillary tangles

APP Amyloid Precursor Protein

MAPT Microtubule associated tau protein

PSEN1 Presenilin1

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