Reviewer #1:

Specific Comments to Authors: In the present review paper, the authors present the "Neural lineage differentiation of human pluripotent stem cells: advances in disease modeling". In detail, the paper and topic is good, but I have some major concerns about the paper which are listed as follows:

o There are several grammatical errors in the text. Please control the text in that manner. The text should be written scientifically. For example, this type of writing is wrong: - Brain diseases affect 1 in 6 people worldwide, ranging from acute neurological diseases such as stroke to chronic neurodegenerative disorders such as Alzheimer's Disease (AD Recent advancements in tissue-engineered brain disease models have allowed us to overcome the shortcomings of animal models, tissue culture models, and epidemiologic patient data that is commonly used to study brain disease; Or, hPSCs include both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs); and etc. Please modify the text.

Response: We appreciate the reviewer's comments. We have edited the paper and removed the grammatical errors. We have changed the abstract to include more scientific writing.

o Where is the "Keywords"?

Response: Thank you so much for pointing out this error. We have included the keywords to the article.

o Table 1, "reference column" should be modified and the name of the authors should be replaced in this column.

Response: Thank you for the comment. We have added names with all references.

o The paper needs a "conclusion" part.

Response: Thank you for the comment. We have included a conclusion part in the manuscript

"Research in hPSCs has proven to be extremely helpful in creating disease models that can corroborate results gleaned from animal models and overcome their associated limitations. Distinct brain cell types can be produced using hPSCs including neurons, astrocytes, oligodendrocytes, microglia, in addition to more advanced heterogeneous systems such as brain organoids. These systems have contributed to the development of models for neurological diseases such as Alzheimer's disease, Parkinson's diseases and many others. Current models that employ hPSCs have certain shortcomings related to the absence of vasculature as well as microglia. However, developing research in the field of tissue engineering that use cocultures, organ-on-chip and assembloids may be able to get around these limitations in the years to come."

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: This manuscript entitled "Neural lineage differentiation of human pluripotent stem cells: advances in disease modeling " did detailed study on hPSCs. The study is interesting. I have some comments for improvement before acceptance.

1 Please write the artical in a regular format, e.g. "Alzheimer's Disease (AD)" in the "abstract"

Response: Thank you for the comment. We have edited the article to follow the format.

2 Some studies have shown that iPSCs can differentiate into microglia, is it possible to provide an overview of microglia in the paper?

Response: Thank you for the comment. We have included a section of microglia in the article.

"**3.1. Microglia**

Although murine models have been the main tool for studying the genetics and function of microglia, there are important distinctions between murine microglia and human microglia when it comes to aging and associated diseases (*70, 71*). Historically, viable microglia cells have been obtained by extracting them from brain tumors or epileptic foci removed from surgery, but this procedure is logistically very challenging. These hurdles were reduced when multiple methods to differentiate microglia from hPSCs were developed (*70-74*). Muffat *et al* published the first protocol in 2016 by producing microglia-like cells from regular and patient hESCs and hiPSCs. This method used serum-free neuroglial differentiation (NGD) media, which contained various components with concentrations adjusted to biologically match human cerebrospinal fluid. In 2017, Abud *et al* described a two-step method to successfully derive microglia-like cells (iMGLs) from ten different hiPSC lines in 5 wk. The transcriptome profile of the derived iMGLs was strikingly similar to that of both adult human and fetal microglia (*75*). Most microglial directed differentiation protocols involve hematopoiesis (*73, 75, 76*). Some reported studies use chemically-defined protocols to generate human microglia through the formation of myeloid progenitors in 30 days (*77*). The Iba-1 protein (Ionized calcium binding adapter molecule 1), a protein that belongs to the calcium-binding protein family, is one of the main markers of microglia (*78*). They are primarily involved in rearranging cytoskeleton and have been used as a marker for three-dimensional reconstruction of microglial cells (*79*). Other general markers used for microglial identification are CD45 and CX3CR1. In a recent study, Dräger *et al* described an effective 8-day protocol for generating induced-transcription factor microglia-like cells (iTF-Microglia) based on the inducible expression of six transcription factors (Human MAFB, CEBP, IRF8 PU.1, CEBP, and IRF5) (*80*)."

3 The relationship between brain disease and stroke is mentioned in the abstract, but the text deals with stroke, can you add some review of hPSCs and stroke?

Response: Thank you for the comment. We have included references related to stroke modeling using iPSCs throughout the article.

"When it comes to *in vitro* stroke modeling, ischemia-like conditions can be simulated by replacing normal O2/CO2 conditions with N2/CO2 and subjecting cells to glucose deprivation (*44*). However, cultures in 2D cannot effectively model stroke due logistical difficulties in restricting oxygenation as well as maintaining nutrition deprivation. However in 2021, Wevers *et al* used neurovascular unit on-a-chip which included a triculture of brain vascular cells, hiPSC-astrocytes and hiPSC-neurons to model ischemic stroke(*45*)."

"Cortical organoids were also used in stroke modeling to study the effects of oxygen-glucose deprivation (OGD), neuronal death that followed, and damaged neural networks(*95*). These models use 2-8 h of OGD with O2 0.1%, CO2 5%, N2 95% gas levels and deoxygenated glucose-free medium to induce ischemia(*95*). It has also been discovered that hypoxic conditions can reduce the number of progenitors and impair the differentiation of immature neurons during the development stage of brain organoids(*96, 97*)."