CHARACTERIZATION OF AUTOFLUORESCENCE AS AN INDICATOR OF ACTIVATION STATE IN NEURAL STEM CELLS

Rachma Khairun Nisaa [1*]

[1]Universitas Muhammadiyah, Surabaya, Indonesia

[*] Corresponding author : rachma9703@gmail.com

ABSTRACT

Recent advancements in stem cell research have uncovered a novel autofluorescence marker pivotal for investigating the dormant state of stem cells. This marker presents a groundbreaking opportunity to monitor the transition of stem cells from a quiescent to an active state, facilitating the identification of cells entering the cell cycle. The primary objective of this research is to comprehensively review this marker's efficacy with the aim of developing therapeutic strategies for generating human nerve cells. A systematic literature search initially yielded 2297 articles on autofluorescence characterization as an indicator of activation state in neural stem cells (NSCs). However, only three articles met the stringent inclusion criteria, underscoring the novelty and scarcity of research in this domain. Autofluorescence, particularly in NSCs, offers a non-invasive approach to studying molecular processes and discerning various activation states, obviating the need for external labels. This technique not only preserves the intrinsic properties of cells but also circumvents biases inherent in traditional labeling methods. Moreover, when coupled with cutting-edge technologies such as Optical Coherence Tomography with Spectral Inverse Analysis (OCSI), it enables precise, real-time monitoring of metabolic alterations in NSCs during their transition from dormancy to activity

Keywords : Autofluorescence; activation; neural stem cells

INTRODUCTION

NSCs are crucial cells in the nervous system responsible for the formation of the nervous system during early development. Although they still exist in small numbers in adults, most of them are in an inactive state (Zhao & Moore, 2018). Somatic stem cells, including adult neural stem cells (NSCs) in the subventricular zone of rats, are in an inactive state in many adult tissues. However, they can be activated as needed to produce new differentiated cells and contribute to tissue function, such as in the reorganization of neural circuits and essential cognitive and behavioral functions (Doetsch et al., 1999; Kriegstein & Alvarez-Buylla, 2009; Bond et al., 2015; Lledo & Valley, 2016; van Velthoven & Rando, 2019; Harada et al., 2021).

In recent decades, research and procedures related to neurogenesis, the process of forming new nerve cells, have made significant advancements (Zhang & Jiao, 2015). During this period, various molecular biomarkers have been discovered. These molecular biomarkers are specific molecules that can be used to identify and study the characteristics and activities of certain cells. In this context, these biomarkers are very useful for investigating neural stem cells (NSCs), both in the embryonic stage and in adult organisms. The nature of stem cells or neural precursors present in the subgranular zone (SGZ) within the hippocampus, an area of the brain involved in the processes of memory formation and learning, is that they are generally considered "quiescent", meaning

they are inactive or not performing many activities under normal conditions. However, despite this, these cells can be activated or become active by various types of stimuli, both from within the body (internal) and from the external environment (external). This indicates that although they may be inactive by default, they have the ability to become active according to specific needs or stimuli. The ability of stem cells or neural precursors to be activated is important in the context of neurogenesis, where the process of forming new neurons can be triggered by the activation of these cells (Duan et al., 2008; Vadodaria & Gage, 2014).

Adult neurogenesis, or the formation of new neurons in adults, begins with progenitor cells, which are early cells capable of developing into neurons. This process goes through several distinct stages, around six stages, marked by changes in the characteristics of the cells involved. The initial stage, referred to as type-1 cells, is the stage where progenitor cells initially appear. Then, these cells undergo a series of changes and divisions to become type-2a, type-2b, and type-3 cells, with each stage marking progress in the differentiation and development of these cells. Finally, these cells develop into immature and mature cells, with each stage indicating different levels of maturity (Kempermann et al., 2004; Zhang & Jiao, 2015).

The first stage, referred to as stage 1, involves type-1 cells, which are neural stem cells resembling radial glia but with different morphology (Fukuda et al., 2003). These cells express the glial fibrillary acid protein (GFAP) and nestin, markers for astrocytic cell types. In stages 2 through 4, type-1 cells are assumed to undergo asymmetric division, giving rise to daughter cells called type-2 cells (Kronenberg et al., 2003). Subsequently, these type-2 cells will develop into three types of presumed transient progenitor cells, which have the capacity for proliferation, specific morphology, and undergo gradual neural differentiation. In stage 5, after going through the previous three stages, these cells are induced to exit the cell cycle and enter a transient postmitotic stage, where they undergo early development into immature neurons. These neurons can be identified by the doublecortin (DCX), NeuN, and Ca2+ calretinin-binding proteins (Li et al., 2002). Finally, in stage 6, these cells become mature new granule cells, expressing calbindin (Jessberger & Kempermann, 2003).

GFAP is a protein involved in the formation of filaments between astrocytes, which are a type of glial cell or support cell in the nervous system. These filaments provide mechanical strength to astrocytes, helping them maintain structure and support for the neurons around them. GFAP also serves as a marker or indicator for active astrocytes or those involved in the process of neurogenesis. Research has shown that astrocytes containing GFAP can act as the origin of new neurons during adult neurogenesis. This suggests that astrocytes, glial cells typically responsible for supporting and protecting neurons, also have the ability to participate in the process of forming new neurons. Increasing research evidence indicates that progenitor cells containing GFAP have the ability to generate specific types of neurons during neurogenesis. (Kriegstein & Götz, 2003).

In a recent study, it was reported that an autofluorescence marker has been discovered which can be utilized to study the inactive state of stem cells. Through this novel marker, the status of stem cells transitioning from a quiescent state to activation can be identified by their activity to enter the cell cycle. This research aims to conduct a review of the latest marker from a recent study to generate human nerve cells, thereby enabling the determination of appropriate therapies.

MATERIALS AND METHODS

In this research, we employed a systematic review method to identify, analyze, and synthesize relevant studies on "Characterization of Autofluorescence as an Indicator of Activation State in Neural Stem Cells." To obtain literature that meets the study's requirements, inclusion and exclusion criteria were established. Comprehensive literature searches were conducted using several major academic databases such as PubMed. Keywords used in the search included "autofluorescence," "activation," "stem cells," and "neural stem cell." Studies identified through the initial search were screened based on their titles and abstracts to identify those that potentially met the inclusion criteria. Findings from this literature review are presented in a structured format, following recognized reporting guidelines such as PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Chosen studies undergo a thorough evaluation based on the inclusion and exclusion criteria set forth in the protocol. These criteria encompass topic relevance, type of study, publication year, and methodological quality. Only those studies that satisfy these criteria are incorporated into the review.

Out of the articles selected and the data extracted, 3 met the inclusion criteria, while 2294 did not and were excluded.

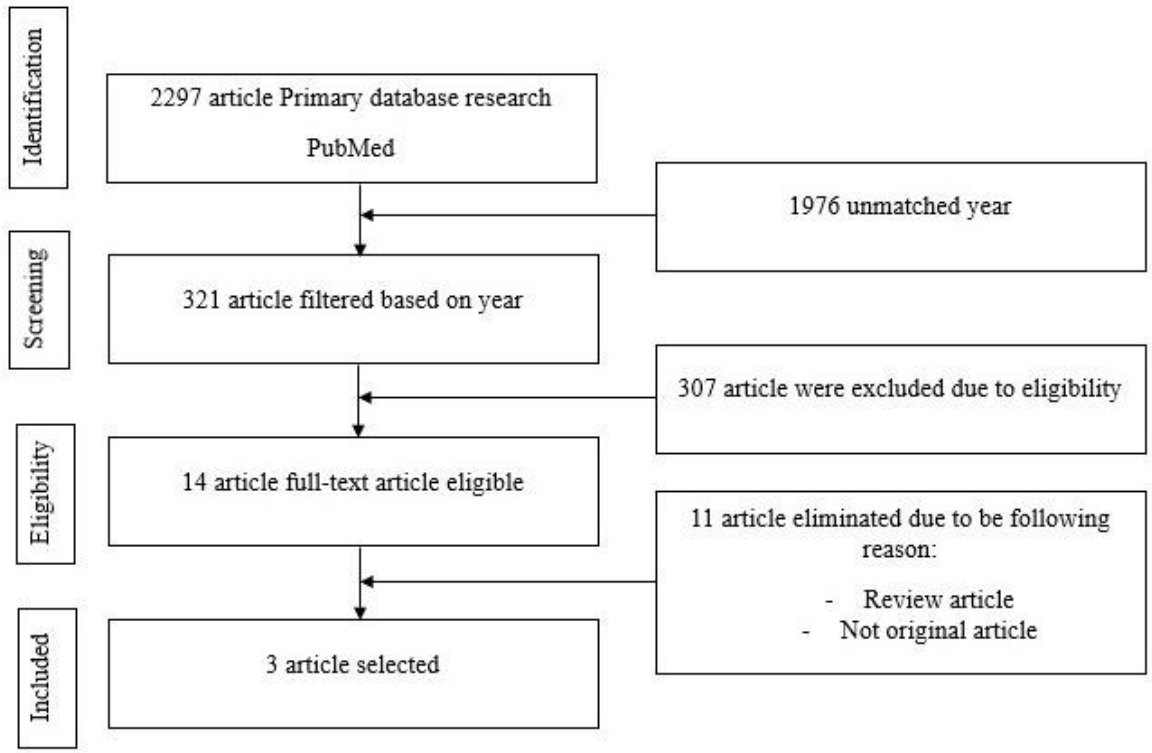

Figure 1. A diagram showing the steps of the systematic review

RESULT AND DISCUSSION

Autofluorescence is a technique that allows direct observation of molecular processes in living cells without requiring additional labels (Bertolo et al., 2020). This means that autofluorescence does not disturb the cells or require the addition of external chemicals that could affect the observation results. This technique also avoids the biases often present in other methods using canonical markers. For example, markers such as intermediate filaments (nestin, vimentin, and GFAP) are often used to identify NSCs in the brain (Leeman et al., 2018; Morrow et al., 2020). However, the expression of these proteins differs significantly between dormant NSCs (qNSCs) and active NSCs (aNSCs) (Morrow et al., 2024).

The autofluorescence of NSCs provides a platform to identify more subtle substates of these cells. For instance, in timelapse observations, autofluorescence allows the identification of NSCs transitioning between dormant and active states, as opposed to those that are still dormant or fully active. In in vivo research, the intensity of autofluorescence correlates with dormant and active substates. With technological advancements, NSC autofluorescence can be used to more precisely identify NSC substates both in vitro (in cell culture) and in vivo (in living organisms) as NSCs exit dormancy and differentiate. Additionally, the intensity of autofluorescence can be used to predict underlying gene expression and cell behavior (Morrow et al., 2024).

Cells can be studied in their natural state without the need for significant destruction or alteration. This technique is also scalable, meaning it can be applied to various types of cells and different research contexts. Additionally, because autofluorescence does not require the addition of external labels (label-free), it eliminates potential biases that might occur with traditional labeling techniques. One of the most potent autofluorescent markers in NSCs is the high level of autofluorescence generated by PAF (Polyunsaturated Aldehyde Fluorescence), which localizes to a subset of lysosomes within these cells. The intensity of this PAF is sufficient to predict the activation state of NSCs without needing additional external markers (Morrow et al., 2024).

Lysosomes in dormant cells (qNSCs) have a broad absorption and emission spectrum, making it challenging to identify specific molecules causing the autofluorescence (Datta et al., 2020). Lysosomes have unique enzymes that enable them to degrade proteins and lipids through endocytosis or lipophagy processes (Thelen & Zoncu, 2017). In qNSCs, which use fatty acid oxidation to generate energy, the ability of lysosomes to break down lipids and provide these fatty acids is crucial for cellular lipid metabolism (Knobloch et al., 2017).

OCSI (Optical Coherence and Scatter Imaging) is a technique used to measure changes in lysosomal and mitochondrial metabolism (Karamata et al., 2005). This technique serves as a dynamic live reporter, providing real-time information about the metabolic changes underlying the transition of cells from dormant to active states (Gardner et al., 2019). It allows researchers to monitor and understand the complex metabolic dynamics in neural stem cells in a more detailed and non-invasive manner.

SUMMARY

Autofluorescence, particularly within neural stem cells (NSCs), presents an invaluable approach for studying intricate molecular processes and discerning various cellular substates, notably the transition between dormant and active states. This technique enables the observation of cells in their native conditions, circumventing the need for substantial manipulation or external labeling, thereby offering insights into cellular behaviors, gene expressions, and metabolic dynamics. Moreover, its scalability across different cell types and research contexts, coupled with its label-free nature, mitigates potential biases inherent in conventional labeling methods. Additionally, the text highlights the complementarity of autofluorescence with techniques such as Optical Coherence and Scatter Imaging (OCSI), which provides real-time data on metabolic alterations, enhancing our understanding of cellular transitions with greater detail and minimal invasiveness.

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