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*Research Article*

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# Molecular Characterization of Transform Kidney Cell Culture Derived From *Macaca fascicularis*

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**ABSTRACT**

**Background:** Non-human primates have a play role as animal model for biomedical research because their similarity of genetic and physiology to human. Our previous study has developed transformed kidney cells from *Macaca fascicularis* (Mf) for in vitro model and vaccine development. The characterization of transformed kidney cells from Indonesia Mf has never been done.

**Aims:** The aim of this study is to characterization by molecular technique of Mf transformed kidney cell with PCR technique. Transform to immortalized cell lines provide a consistent and readily available source of cells for research use.

**Methods:** We analyzed CD46 and CD155 as receptors for measles and poliovirus, respectively; endosialin, CD24, and vWF as cellular morphology markers (fibroblast, epithelial, and endothelial cells); and PCNA and P53 as markers of cell proliferation and tumor suppression gene using PCR technique.

**Results:** We have been successfully to characterized all the target genes above on transformed kidney cell from *Macaca fascicularis*, Vero cells, and untransformed kidney cells. This study shown based on molecular study our transformed kidney cells showed consistent characteristic to their source of cells.

**Conclusion:** Our research suggests that our transformed kidney cells from *Macaca fascicularis* have potency as in vitro model and vaccine development at molecular level.

## INTRODUCTION

Kidney cells are widely used in biomedical research as in vitro drug candidates and are characterized by their inability to produce interferons (Yoshikawa et al., 2006). This property makes kidney cells valuable in biomedical studies for investigating virus host cell interactions and assessing antiviral drug efficacy (Sandler et al., 2014). These cells are also employed in the production of viral vaccines, such as polio and rubella vaccines, as they efficiently support viral replication (Desmyter et al., 1968). The kidney cells predominantly used in biomedical applications are Vero cells, derived from the kidney epithelial cells of the African green monkey (*Chlorocebus sabaeus*), which have been developed into four subtypes: Vero JCRB0111, Vero CCL-81, Vero 76, and Vero E6 (Konishi et al., 2022).

Primary kidney cell culture consists of cells directly isolated from the kidney tissue of a living organism and cultured in vitro without undergoing transformation or

immortalization processes (Capes-Davis et al., 2021). Previous research conducted by Yusni et al ((2023) described the primary culture of kidney cells derived from the Indonesian long-tailed macaque (*Macaca fascicularis*) as a cellular model for evaluating vaccine potential and drug efficacy,demonstrating comparable capabilities to Vero cells. Kidney cell cultures are used for viral susceptibility testing (e.g., poliovirus and measles virus) using various assays, including the serum neutralization test, cytological examination, and plaque assay (Cai et al., 2024).

Furthermore, the development of this indigenous cell line aligns with the global and national agenda for sustainable development. Establishing a characterized cell line from Indonesian *Macaca fascicularis* represents a shift towards a knowledge-based economy, directly supporting SDG 9 (Industry, Innovation, and Infrastructure). By enhancing scientific research and upgrading technological capabilities in the domestic pharmaceutical sector, this study aims to reduce dependency on imported biological materials (such as Vero cells) and foster national independence in vaccine raw materials.

This research aims to characterize the expression of CD46 (cluster of differentiation)46, the receptor for measles virus, and CD155 (cluster of differentiation)155, the receptor for poliovirus binding; endosialin as a fibroblast cell marker; von Willebrand factor (vWF) as an endothelial cell marker reflecting kidney function; and CD24 as an epithelial cell marker. Additionally, this research evaluates the gene expression of PCNA (proliferating cell nuclear antigen) and P(Protein)53, which serve as genetic markers for cell proliferation.

## MATERIALS AND METHODS

### Mf Kidney Cell Culture

Experimental procedures involving animals were approved by the PSSP-IPB Animal Ethics Committee (approval number PRC-20-B-002) and conducted at the Lodaya Animal Research Facility of PSSP-IPB, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Kidney samples used in this study were obtained from a previous study by Yusni et al. (2023) and originated from healthy adult long-tailed macaques aged 4–6 years. Kidney collection was performed aseptically by a licensed veterinarian to ensure sterility and tissue viability. Isolated kidney tissues were processed to establish primary kidney cell cultures and maintained under standard cell culture conditions. The primary kidney cell cultures were successfully subcultured up to passage 6. Cells used in subsequent experiments were initiated at passage 3, corresponding to a phase of optimal cell viability and proliferative capacity.

### Mf Kidney Cell Transformation

Transformation of Mf kidney cells was performed using a DNA-containing virus following the in vitro transformation protocol described by Dulbecco. Primary Mf kidney cells at passage 3 were seeded into 6-well culture plates and cultured until approximately 80% confluence was achieved. Cells were then exposed to a transforming agent containing adenovirus at a multiplicity of infection (MOI) of 5–10 for 24 hours in serum-free medium. After incubation, the medium was replaced with a complete growth medium supplemented with 10% fetal bovine serum (FBS), and cells were further cultured for 7–10 days. Morphological changes indicative of cellular transformation, including loss of contact inhibition, multilayered colony formation, and increased proliferation rate, were monitored and documented using an inverted microscope. Stable transformed kidney cell lines were maintained in DMEM supplemented with 10% FBS and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) and subcultured every 3–4 days. Successful transformation was confirmed by PCR amplification of the antigen gene and analysis of molecular markers associated with cell proliferation and tumor suppressor activity (Table 1).

### *mRNA Extraction and cDNA Synthesis*

Total mRNA was extracted using the RNeasy Mini Kit according to the manufacturer's instructions (Table 2). RNA concentration and purity were measured using a NanoDrop™ One spectrophotometer. For cDNA synthesis, 10 ng/µL of total RNA was reverse transcribed using the SensiFAST cDNA Synthesis Kit. Reverse transcription reactions were performed in a thermal cycler under standardized cycling conditions, and the resulting cDNA was stored at –20°C until further analysis.

### cDNA Amplification by Polymerase Chain Reaction

PCR amplification was performed to detect specific marker genes used as biomarkers for kidney cell cultures. Seven pairs of forward and reverse primers were specifically designed for this study. PCR reactions were prepared using Bioline Red Mix and conducted under defined thermal cycling conditions. Amplified products were separated by agarose gel electrophoresis and visualized using the Gel Doc 2000 Gel Documentation System.

**Visualization of PCR Results Using Agarose Gel Electrophoresis**

The quality test of the stock DNA was subsequently conducted through DNA electrophoresis of the stock using 1% agarose gel. A total of 2 µL of the sample was mixed with 1 µL of loading dye. Electrophoresis was performed for 35 minutes at a voltage of 100 volts. Visualization of the electrophoresis fragments was performed using ethidium bromide (EtBr) staining and observed under UV light with a Gel Doc 2000 documentation.

## RESULTS AND DISCUSSION

Cell transformation in this study was performed using an adenoviral-mediated intervention that had been previously established and optimized, as illustrated in Figure 1. Adenoviral vectors are widely used in cell transformation studies due to their high transduction efficiency, broad host cell tropism, and ability to mediate stable gene delivery without integrating into the host genome. Following viral intervention, microscopic observations demonstrated that the virus became fully incubated throughout the culture by the second passage, indicating successful viral propagation and stable adaptation within the cell population (Figure 1b). The uniform distribution of viral effects across the culture suggests that the transformation process did not induce overt cytotoxicity and that the cells retained their viability and proliferative capacity after viral exposure. This observation is particularly important, as excessive viral cytopathogenic

**Table 1.** Genes used for PCR amplification

| Marker (5'- 3') | Nucleotide Primer Sequences | References | Annealing Temp. (°C) | Amplicon |
|---|---|---|---|---|
| *CD46* Forward | TGGCTACCTGTCTCAGATGAC | www.Origene.com | 55 | 115bp |
| *CD46* Reverse | GCATCTGATAACCAAACTCGTAAG | | | |
| *CD155 Forward* | CTGAGGATGTTCG GGTTGCG | Chapel et al., 2018 | 57 | 149bp |
| *CD155* Reverse | GTGAGCTGGACCTTCTGAACC | | | |
| *Endosialin* Forward | GTGAGCTGGACCTTCTGAACC | www.Origene.com | 57 | 126bp |
| *Endosialin* Reverse | TCCAGCAACTCATCTCCGAGGT | | | |
| *VWF* Forward | CCTTGAATCCCAGTGACCCTGA | www.Origene.com | 55 | 157bp |
| *VWF* Reverse | GGTTCCGAGATGTCCTCCACAT | | | |
| *CD24* Forward | CCCACCAGATTTATICCAG | Panagiotou et al., 2022 | 55 | 255bp |
| *CD24* Reverse | GACTCCAGACGCCATITG | | | |
| *PCNA* Forward | CCTGCTGGGATATTAGCTCCA | Bai et al., 2021 | 55 | 109bp |
| *PCNA* Reverse | CAGCGGTAGGTGTCGAAGC | | | |
| *P53* Forward | AATCATCCATTGCTIGGGACG | Darusman et al., 2022 | 57 | 255bp |
| *P53* Reverse | TAGAGACGGCTCTTCTGCC | | | |

**Table 2.** RNA Concentration of transformed kidney cells

| Cell Type | ng/mL | A260/A280 | A260/A230 |
|---|---|---|---|
| Vero cells | 337,2 | 2,03 | 2,28 |
| Normal kidney cells | 24,9 | 2,11 | 1,53 |
| Immortal kidney cells | 43,4 | 2,04 | 1,19 |

effects could compromise downstream molecular analyses and the functional utility of the established cell line.

This research was conducted to analyze the expression of genes involved in viral receptor recognition, tumor suppression, stromal and endothelial identity, and cellular proliferation. These molecular markers were selected to provide a comprehensive characterization of *Mf* progenitor kidney cell cultures following adenoviral transformation. Such characterization is essential to evaluate whether the transformed cells retain key molecular and functional features of their parental cells, which is a critical requirement for their application as reliable in vitro models. The molecular profiling performed in this study contributes to a deeper understanding of *Mf* kidney cell development and supports their potential use in biomedical research, particularly in in vitro testing platforms, virological studies, and vaccine development. The results demonstrated that the genes CD46, CD155, endosialin, CD24, vWF, PCNA, and P53 were successfully amplified in *Mf* progenitor kidney cell cultures. Importantly, these cultures exhibited molecular characteristics consistent with those of their parental kidney cells, reinforcing their suitability as sustainable in vitro models for translational biomedical applications.

The isolation of high-quality RNA is a critical prerequisite for accurate gene expression analysis. In this study, total mRNA was successfully isolated from kidney cells, and the quality and quantity of RNA were evaluated using NanoDrop spectrophotometry. The RNA concentrations obtained are summarized in Table 2. Overall, the isolated RNA exhibited purity values ranging from 1.8 to 2.0, with concentrations exceeding 100 ng/µL, indicating that the RNA samples were of sufficient quality for downstream molecular analyses, including cDNA synthesis and PCR-based gene amplification. According to established criteria, A260/A280 ratios below 1.8 are indicative of protein or polysaccharide contamination, whereas ratios above 2.0 may suggest DNA contamination. Similarly, suboptimal A260/A230 ratios are associated with contamination by organic compounds such as phenol or residual chaotropic salts. In the present study, although minor variations in purity ratios were observed, all RNA samples met the minimum quality requirements for reliable molecular characterization.

Amplification of the CD46 gene in transformed *Mf* kidney cells yielded a DNA band of 115 bp, as shown in Figure 2. This result confirms the successful detection of the CD46 gene, which serves as a receptor for the measles virus. CD46 is a complement regulatory protein expressed on the surface of epithelial cells and is known to function as a receptor for both wild-type measles virus and measles vaccine strains (Lok et al., 2018). The detection of CD46 expression in both established and transformed *Mf* kidney cells indicates that the transformed cells retain key epithelial and viral receptor properties comparable to their parental counterparts. These findings are consistent with previous studies reporting CD46 expression in a wide range of cell types, including epithelial cells, lymphocytes, macrophages, and neurons. Notably, CD46 has been described as a "pathogen magnet," acting as an entry receptor for multiple bacterial and viral pathogens (Dhiman et al.,

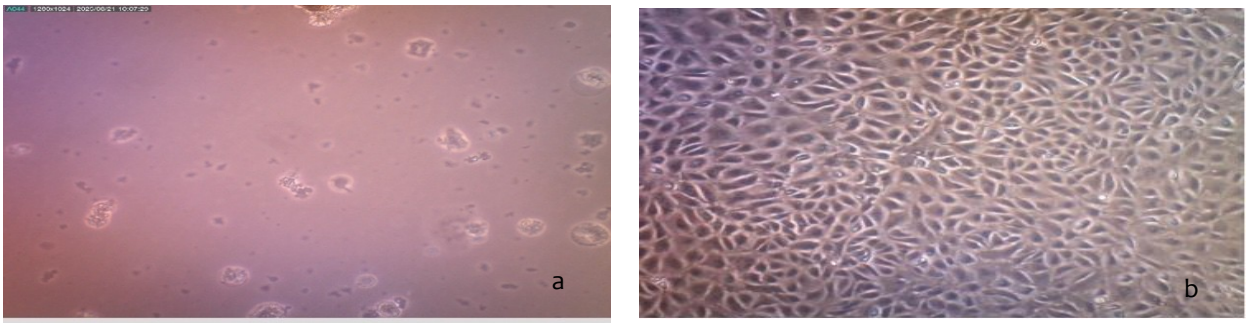


**Figure 1.** Morphologies of transformed cells grown (a) as adherent culture in T-flasks or as suspension culture (b) Morphology of transformed cells at passage 2.

2004). The presence of CD46 in the established kidney cells highlights their potential utility in virological research and vaccine-related studies.

The CD155 gene, a known poliovirus receptor, was also successfully amplified, producing a DNA band of 149 bp (Figure 2). CD155 encodes an immunoglobulin superfamily adhesion molecule involved in cell adhesion, migration, proliferation, and immune modulation (O'Donnell et al., 2020). Its role as a poliovirus receptor underscores its importance in virology and vaccine development. In this study, CD155 expression was detected in established transformed *Mf* kidney cells, Vero cells, and normal kidney cells, with comparable expression patterns observed across all cell types. These findings indicate that the transformed Mf kidney cells maintain CD155 expression at levels similar to those of established commercial cell lines. Previous studies have demonstrated that kidney progenitor cells retain parental characteristics and exhibit continuous proliferation, a feature that parallels the behavior of cancer cells. Importantly, while CD155 expression facilitates viral binding, it does not inherently confer susceptibility to uncontrolled viral replication. Furthermore, reduced CD155 expression has been associated with decreased tumor cell migration, highlighting its broader relevance in cancer biology (Molfetta et al., 2020).

The expression of the endosialin gene was confirmed by the presence of a 126 bp DNA band following agarose gel electrophoresis (Figure 3). Endosialin, also known as CD248, is a transmembrane glycoprotein expressed in mesenchymal stromal fibroblasts and pericytes during tissue development, angiogenesis, and tumor progression (Lu et al., 2018).

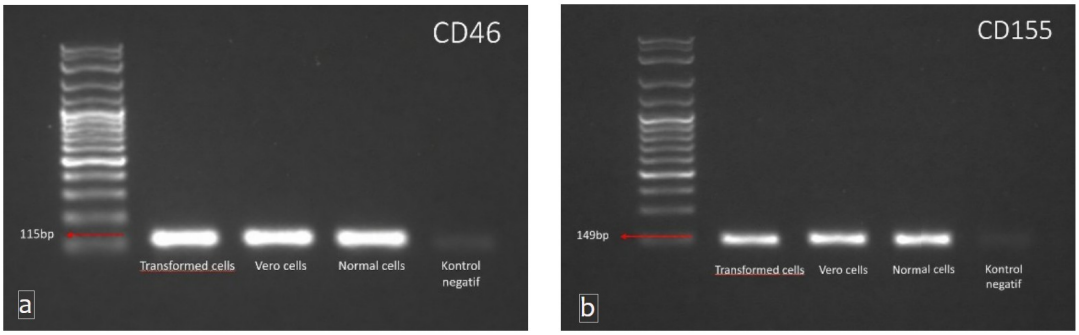


**Figure 2.** The electropherogram of CD46 (a ) and CD155 (b) gene showing amplification in transformed *Mf* kidney cells, Vero cells, and normal kidney cells. This result suggest transformed *Mf* kidney cells have a viral receptor.

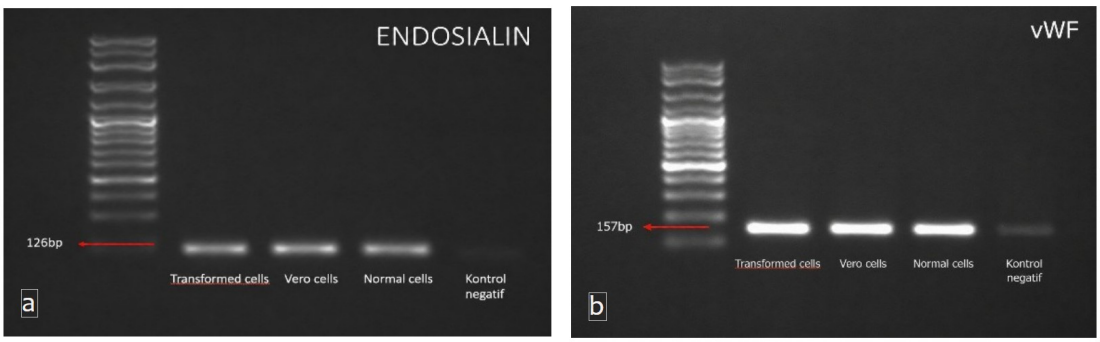


**Figure 3.** Amplification of the endosialin and vWF gene, a kidney cells marker have identified in transformed *Mf* kidney cells, Vero cells, and normal kidney cells. The amplification show transformed *Mf* kidney cells has a potency as in vitro kidney model**.**

In the context of kidney biology, endosialin serves as a marker of fibroblast activation and fibrosis (Y. Hong et al., 2023). The detection of endosialin expression in transformed *Mf* kidney cell cultures, as well as in Vero and normal cells, indicates that the transformation process did not abrogate stromal-associated gene expression. Endosialin is known to potentiate TGF-β signaling by stabilizing its receptor, thereby enhancing fibroblast proliferation and extracellular matrix production (Y. K. Hong et al., 2024). Beyond its role in cancer, endosialin is implicated in tissue repair and fibrotic processes, including kidney fibrosis, liver fibrosis, adipose tissue fibrosis, atherosclerosis, and wound healing (Hu et al., 2021; Kondo et al., 2022). The Endosialin plays an important role in tissue remodeling, fibroblast proliferation, and cellular microenvironment interactions. The presence of this marker suggests that the transformation process did not completely eliminate the stromal identity of the original cells, thereby preserving the biological relevance of the kidney cell culture.

The vWF gene, an established endothelial cell marker, was successfully detected by the presence of a 157 bp DNA band (Figure 3). vWF encodes a glycoprotein essential for primary hemostasis and stabilization of factor VIII during coagulation (Cortes et al., 2023). In kidney cells, vWF serves as a biomarker of endothelial injury and is often elevated in chronic kidney disease and inflammatory states. The detection of vWF in transformed *Mf* kidney cells, Vero cells, and normal kidney cells indicates that endothelial-associated molecular features are maintained across these cell types. Abnormal vWF synthesis and activity, particularly in the context of uremic toxins and metabolic dysregulation, can impair platelet adhesion and contribute to bleeding complications (Noone et al., 2018). The vWF is an endothelial marker associated with vascular function and blood vessel homeostasis. The presence of this marker suggests that the cultured cells still reflect certain physiological properties of kidney tissue, particularly those related to vascular and endothelial functions. The presence of vWF expression in the established kidney cells further supports their relevance as in vitro models for studying vascular and endothelial aspects of kidney pathology.

Amplification of the CD24 gene produced a 255 bp amplicon, confirming its expression in the analyzed samples (Figure 4). CD24 is a glycosylphosphatidylinositol-anchored cell surface protein widely used as an epithelial marker in cancer

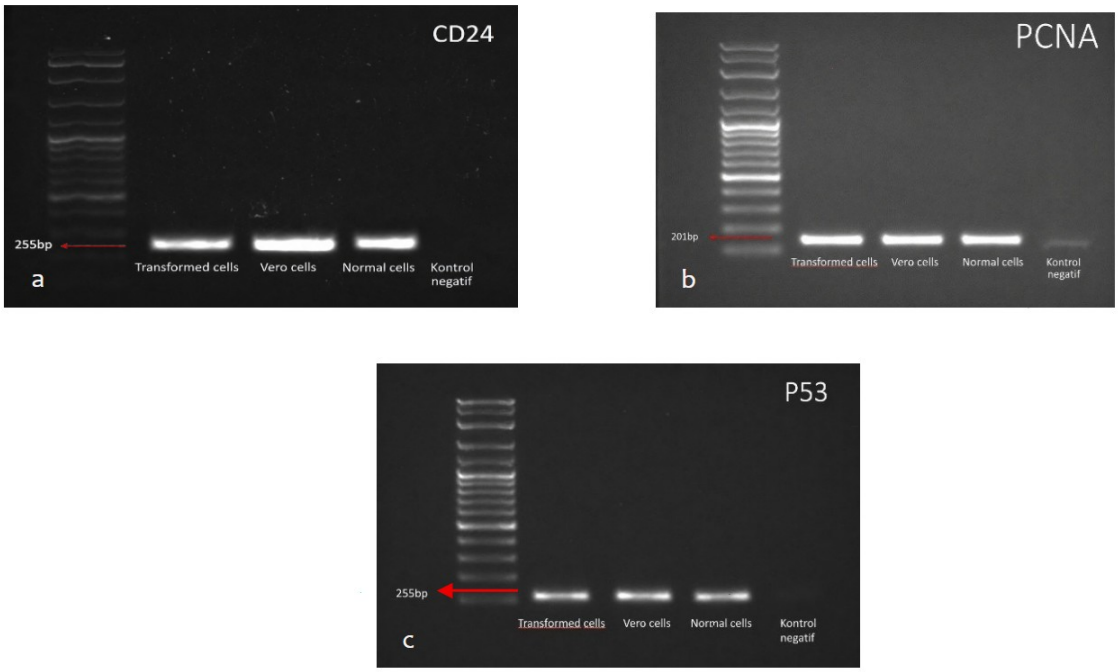


**Figure 4.** Agarose gel visualization of CD24 (a), PCNA (b), and P53 (c) gene amplification in the samples of transformed *Mf* kidney cells, Vero cells, and normal kidney cells. The proliferation gene was successfully detected in the transformed *Mf* kidney cells.

and stem cell research. It plays a critical role in epithelial differentiation and barrier function by regulating tight junction proteins such as ZO-1, occludin, and claudins (Balla, 2009). The presence of CD24 expression in both transformed *Mf* kidney cells and normal kidney cells indicates that epithelial identity is preserved following transformation. Notably, previous studies have identified a rare population of CD24-expressing kidney epithelial cells capable of regenerating tubular structures after injury (Shrestha et al., 2023). CD24 is an epithelial marker involved in cell differentiation, cell adhesion, and kidney tissue regeneration. The persistent detection of CD24 suggests that the transformed cells still represent the original kidney tissue characteristics, supporting their potential use as a relevant in vitro model. The retention of CD24 expression in long-term *Mf* kidney cell cultures suggests that these cells possess regenerative potential and differentiated epithelial characteristics comparable to those of widely used commercial kidney cell lines, including Vero cells (King et al., 2012).

Amplification of the PCNA gene resulted in a 109 bp amplicon, confirming the presence of this proliferation marker in the analyzed samples (Figure 4). PCNA is a critical component of the DNA replication machinery, functioning as a processivity factor for DNA polymerase (Zheng et al., 2020). It is widely used as a marker of cell proliferation and plays an important role in the regulation of chronic kidney disease progression. PCNA functions as a proliferation marker involved in DNA replication and cell cycle regulation.

The persistent detection of PCNA demonstrates that the transformed cells maintain stable and continuous growth potential, supporting their suitability for long-term cell culture applications in in vitro research and vaccine development. The detection of PCNA expression in transformed *Mf* kidney cells, Vero cells, and normal cells indicates active proliferative capacity across these cell types. Previous studies have reported PCNA expression in both progenitor and normal kidney cells, supporting its role in cellular renewal and tissue maintenance. Given its central role in DNA replication, stable PCNA expression is essential for controlled cell proliferation and genomic integrity (Zhao et al., 2012).

Amplification of the *P53* gene produced a 255 bp amplicon, corresponding to the expected molecular weight (Figure 4). *P53* encodes a tumor suppressor protein commonly referred to as the "guardian of the genome" due to its pivotal role in maintaining DNA integrity (Zhang et al., 2024). Beyond tumor suppression, P53 is involved in cell development, aging, and differentiation. The presence of P53 expression in both transformed *Mf* kidney cells and normal kidney cells indicates that key regulatory pathways governing cell cycle control and genomic stability are preserved following transformation. These findings are consistent with previous reports demonstrating P53 expression in kidney progenitor cells and other transformed cell types, including fibroblasts (Garrido-Jimenez et al., 2021). Importantly, the establishment of sustainable *Mf* kidney cell cultures with preserved P53 expression supports their safe and controlled use in biomedical research. This condition suggests that the transformation process did not completely abolish cell cycle regulation or DNA damage surveillance mechanisms. Therefore, the concurrent presence of PCNA and P53 may reflect a balance between proliferative capacity and genomic stability control, supporting the potential use of the transformed cells as a stable and biologically relevant in vitro model.

From a broader perspective, the successful molecular characterization of these transformed *Mf* kidney cells aligns with sustainable development goals, particularly SDG 8, by supporting the development of a domestic biotechnology sector. The availability of locally derived, well-characterized cell lines can reduce dependence on imported biological materials, foster high-skilled employment, and provide cost-effective alternatives for vaccine production and biomedical research. The translation of biological discovery into a viable industrial resource exemplifies the potential of bioprospecting to generate economic value while retaining the benefits of local biodiversity within the nation.

## CONCLUSION

Our study shows that Mf-derived kidney progenitor cells express genes related to kidney function. Despite limited markers and lack of protein analysis, this first characterization demonstrates successful differentiation. These cells show promise as in vitro models and vaccine platforms, highlighting Indonesian primate biodiversity's strategic value and supporting SDG 8 and SDG 9.

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**AUTHORS CONTRIBUTION**

G.I.R: Writing – original draft, Methodology, and Data curation, S.S.M: Writing – original draft, Supervision, Methodology, Resources, Methodology, Investigation. S.M: Methodology, Formal analysis, Writing – review & editing, and Conceptualization. I.I, A.K.K.T, E.D.A: Methodology. U.S: Methodology, Conceptualization. R.R, A.W, W.E: Project administration H.S.D: Project administration, Conceptualization.

*"The author declares that there is no conflict of interest with the parties involved in this research".*

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