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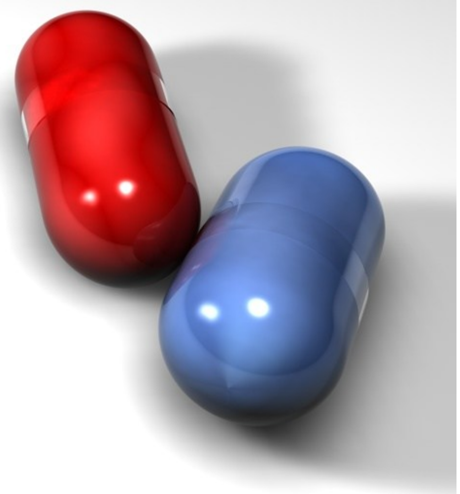

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Letter to the Editor

6-Formylindolo[3,2-b]carbazole promotes proliferation of adipose-derived mesenchymal stem cells via activation of the Ahr–IL-6 signaling axis

Dear Editor,

The tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) is a high-affinity endogenous ligand of the aryl hydrocarbon receptor (Ahr). Ahr has emerged as a promising target in stem cell research — including both mesenchymal stem/stromal cells (MSCs) and hematopoietic stem cells (HSCs) — due to its critical roles in regulating self-renewal, proliferation, differentiation, and immunomodulation, mediated through the regulation of metabolism-related enzymes (e.g., *Cyp1a1, Cyp1b1*) and cytokines (e.g., TGF-β, IL-6) (Yin et al., 2016; Seo et al., 2025). However, the contribution of the FICZ–Ahr–IL-6 network to MSC proliferation re-mains poorly understood.

This study investigates the effect of FICZ on the proliferation of adipose-derived MSCs (AD-MSCs) cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B, 1 ng/mL basic fibroblast growth factor, and 1 ng/mL epidermal growth factor. We compare the fold-change in proliferation and expression of related genes — including *Ahr*, *Cyp1a1*, *Cyp1b1*, and *Il-6* — in AD-MSCs treated with or without FICZ across different cell passages.

Adipose tissues were obtained from healthy female donors (aged 25–30 years) at Hanoi Obstetrics and Gynecology Hospital. AD-MSCs were isolated as described elsewhere (Kim et al., 2013). The concentration of FICZ used (100 nM) was selected based on established protocols from previous reports (Huang et al., 2022; Vrzal et al., 2023). AD-MSCs at passages 1, 2, and 3 (P1, P2, P3) were seeded at a density of 5 × 10^4 cells/well in 24-well plates and cultured in DMEM supplemented with 10%fetal bovine serum, with or without 100 nM FICZ.

Morphological evaluation was performed on day 1, 3, 5, and 7 under an inverted phase-contrast microscope. Representative images from P2 cells (Figure 1, upper row) revealed no visible alterations in fibroblast-like morphology between treated and untreated groups. To assess multipotency, AD-MSCs at P2 were cultured for 21 days in StemMACS differentiation media: AdipoDiff (1 × 10^5 cells/well in 12-well plates), OsteoDiff (5 × 10^4 cells/well in 12-well plates), and ChondroDiff (2.5 × 10^5 cells/tube in 15-mL conical tubes), with or without FICZ. Adipogenic, osteogenic, and chondrogenic differentiation was confirmed by oil red O, alizarin red, and alcian blue staining, respectively.

FICZ-treated cells maintained their differentiation capacity, evidenced by the accumulation of lipid droplets, calcium deposits, and glycosaminoglycans (Figure 1, lower row). Flow cytometry (MACSQuant® VYB, Miltenyi Biotec, Germany) analysis further confirmed that AD-MSCs retained their characteristic surface marker expression following FICZ treatment, including high levels of CD90, CD73, and CD105, and a lack of HLA-DR expression (Table I), indicating preserved immunophenotypic identity. There were no statistically significant differences in the expression of surface markers between untreated and FICZ-treated cells.

Specifically, CD90 expression remained high (99.5% ± 0.6 in untreated vs. 99.0% ± 0.5 in treated cells; $p>0.05$), as did CD73 (99.7% ± 0.3 vs. 99.5% ± 0.4; $p>0.05$) and CD105 (95.5% ± 0.5 vs. 95.1% ± 0.5; $p>0.05$). Both groups showed negligible expression of the hematopoietic marker HLA-DR (0.01% ± 0.01 vs. 0.02% ± 0.01; $p>0.05$). These findings indicate that FICZ treatment does not alter the characteristic surface marker expression of AD-MSCs. Next, to evaluate proliferation, AD-MSCs were harvested at P1, P2, and P3, and viable cells were counted by using the trypan blue exclusion method with a hemocytometer. FICZ promoted the proliferation rate of AD-MSCs, with fold changes of 1.1 ± 0.0, 1.1 ± 0.0, and 1.2 ± 0.0 at P1, P2, and P3, respectively, compared to untreated controls ($p<0.05$) (Figure 2). These results suggest that FICZ significantly promotes the proliferation of AD-MSCs without compromising their phenotype.

It has been reported that Ahr plays a role in stem cell proliferation in a ligand- and context-dependent manner (Larigot et al., 2018). Pharmacologically, FICZ is recognized as a potent, transient agonist of Ahr. To investigate the effect of FICZ on Ahr signaling and downstream targets, the expression of *Ahr*, *Cyp1a1*, and *Cyp1b1* was evaluated in AD-MSCs. Passage 2 AD-MSCs were treated with 10–100 nM FICZ and harvested at 8, 16, 24, and 48 hours for qRT-PCR analysis. At 100 nM, FICZ induced peak gene expression at 24

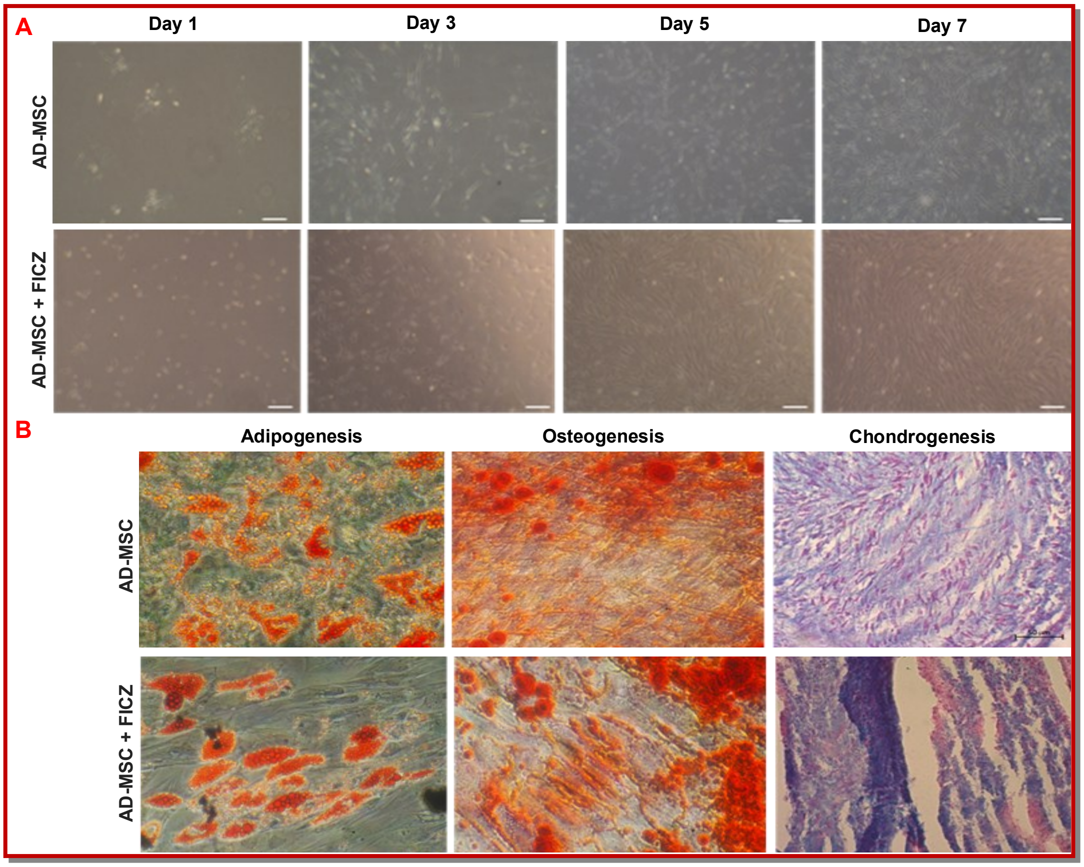

Figure 1: Morphological observation and characterization of AD-MSCs cultured with or without FICZ (100 nM) at passage 2. Phase-contrast microscopy images of AD-MSCs cultured without or with FICZ at day, 3, 5, and 7 (A, magnification 4x). Scale bar: 50 μm. Adipogenesis, osteogenesis, and chondrogenesis differentiation of AD-MSCs. Following adipogenesis, osteogenesis and chondrogenesis differentiation, lipid droplets, calcium deposits and glycosaminoglycans are revealed, respectively (B, magnification 20x)

Table I

%Expression of MSC surface markers and human leucocyte antigen-DR treated with FICZ

CD marker	untreated AD-MSC (%)	FICZ (100 nM)-treated AD-MSC (%)	p value
CD90	99.5 ± 0.6	99.0 ± 0.5	>0.05
CD73	99.7 ± 0.3	99.5 ± 0.4	>0.05
CD105	95.5 ± 0.5	95.1 ± 0.5	>0.05
HLA-DR	0.0 ± 0.0	0.0 ± 0.0	>0.05

Values are mean ± SD; n=3 animals in each group; treatment period was 7 days

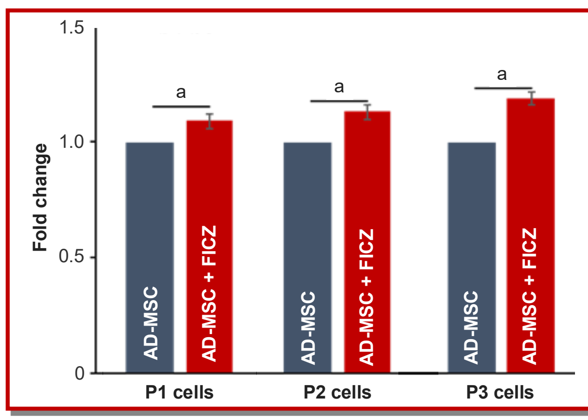

Figure 2: Proliferation of AD-MSCs treated with or without FICZ. Fold change in cell proliferation of FICZ-treated cells compared to untreated cells at passage 1 (P1), P2, and P3. Data are mean ± SD from three independent experiments. [a]p<0.05 vs. untreated control

hours, with *Ahr* upregulated ~3.8-fold (Figure 3A), *Cyp1a1* ~7.1-fold (Figure 3B), and *Cyp1b1* ~9.6-fold (Figure 3C), compared to untreated controls. Given the known role of IL-6 in promoting mesenchymal stromal cell proliferation (Dorronsoro et al., 2020), *Il-6* gene expression was also assessed. FICZ treatment induced a ~3.2-fold increase in Il-6 expression at 24 hours (Figure 3D), coinciding with the transcriptional peaks of *Ahr* and its downstream genes. This finding supports a potential link between *Ahr* activation and IL-6 upregulation in AD-MSCs. Interestingly, we observed significantly higher *Ahr* expression in AD-MSCs cultured in StemMACS MSC expansion medium compared to those in standard DMEM (unpublished data), suggesting the presence of an *Ahr* ligand on the proprietary formulation. Further studies are required to identify the

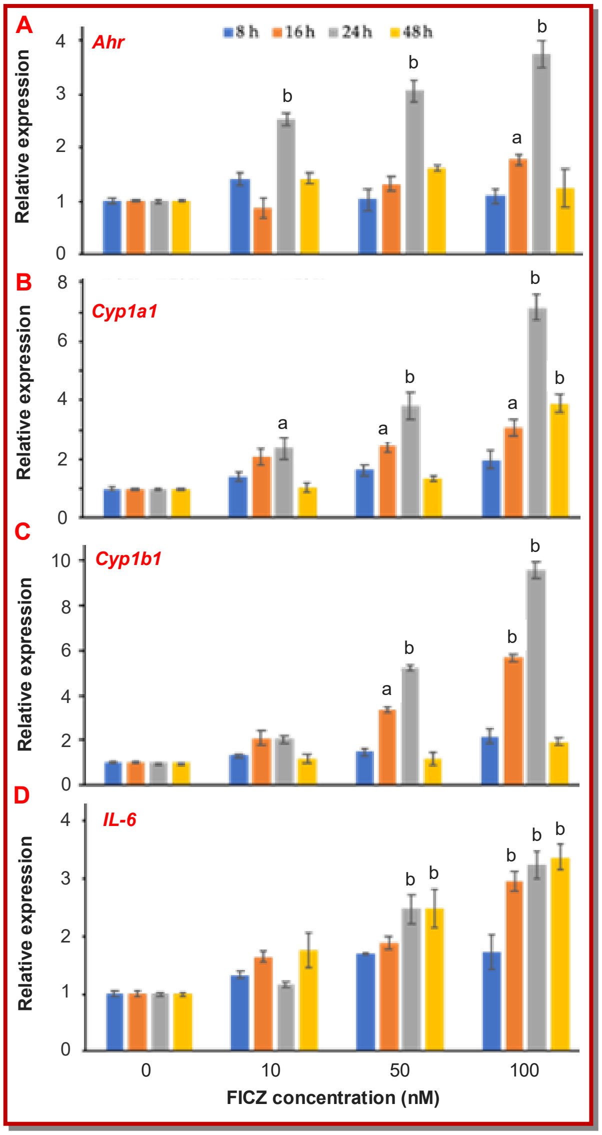

Figure 3: Expression of *Ahr*, *Cyp1a1*, *Cyp1b1*, and *Il-6* in FICZ-treated and untreated AD-MSCs. Gene expression levels were measured by qPCR at 8, 16, 24, and 48 hours. Data are mean ± SD from three independent experiments. GAPDH housekeeping genes were used for normalization. [a]p<0.01, [b]p<0.005 vs. untreated control

specific factors responsible for *Ahr* activation in this medium. A hypothetical model summarizing the proposed interplay between FICZ and *Ahr*-*IL-6* signaling axis in promoting AD-MSC proliferation (Figure 4).

Taken together, present findings suggest that FICZ activates the *Ahr*–*IL-6* signaling axis to enhance the proliferation of AD-MSCs while preserving their multipotency, including the ability to differentiate into adipogenic, osteogenic, and chondrogenic lineages. Importantly, *Ahr* is also expressed in epidermal keratinocytes, where it regulates cutaneous immune responses and homeostasis (Tsuji et al., 2012; Nguyen et al., 2013; Tanaka et al., 2018). Given that FICZ is a photoproduct of tryptophan generated upon UV exposure, its regulatory role in skin-resident cells—including keratinocytes and hypodermal adipose-derived MSCs—warrants further investigation. Elucidating the role of the FICZ–*Ahr*–*IL-6* signaling axis in the skin micro-environment under physiological or light-induced conditions may provide novel insights into tissue regeneration and the treatment of inflammatory and degenerative skin disorders.

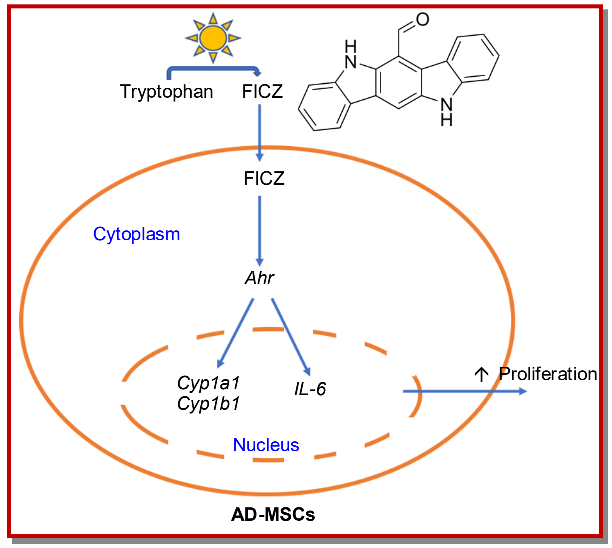

Figure 4: Hypothetical model illustrating the potential role of FICZ and *Ahr-IL-6* signaling axis in promoting AD-MSC proliferation while preserving stemness and differentiation potential. FICZ formed by the action of light on tryptophan also known as an endogenous ligand of *Ahr* can activate *Ahr* signaling in AD-MSCs and lead to the upregulation of downstream target genes such as *Cyp1a1*, *Cyp1b1*, and *Il-6* that may contribute to the maintenance of AD-MSC stemness and differentiation potential, while supporting their proliferation capacity.

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Ethical Issue: Adipose tissues were obtained in accordance with protocol approved by the hospital's ethics committee (Ref. No. 2206 CN/PS).

Conflict of interest: The authors declare that they have no conflicts of interest.

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Thanh Trung Tran[1], Tien Thi Vu[1], Kien Trung Tran[2], Taisuke Nakahama[3], and Nam Trung Nguyen[1]

[1]Institute of Biology, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [2]Hanoi Obstetrics and Gynecology Hospital, Hanoi, Vietnam; [3]Department of RNA Biology and Neuroscience, Osaka University, Suita, Osaka, Japan.

Corresponding authors:

Email: nam@ibt.ac.vn

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