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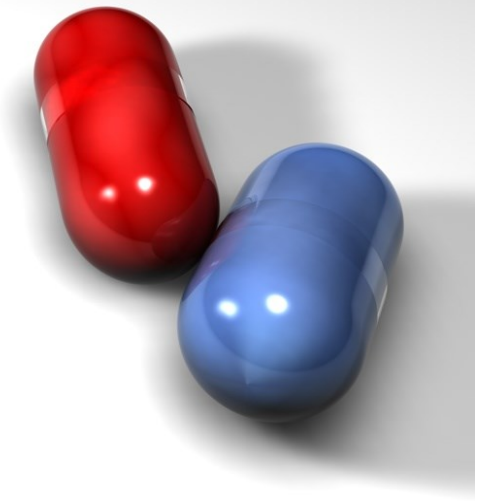

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Letter to the Editor

Bioactive peptides SL-13R and KS-13 enhance human adipose-derived mesenchymal stem cell proliferation *in vitro*

Dear Editor,

The importance of peptide therapeutics in pharmaceutical research is increasing (Akbarian et al., 2022). The use of bioactive peptides to expand stem cells is urgently needed to increase the number of these cells for clinical applications (Wang et al., 2022). It has been reported that the SL-13R peptide enhances the expansion of hematopoietic stem cells (HSCs) *in vitro* (Nii et al., 2021). However, the bioactivity of the SL-13R peptide in adipose-derived mesenchymal stem cells (AD-MSCs) is not known. Therefore, we aimed to examine whether the SL-13R peptide enhances AD-MSC proliferation *in vitro*. Recent studies have shown that some peptides can also promote the proliferation of both HSCs and MSCs (Dayem et al., 2023). Bioactive compounds such as genistein were shown to be potent stimulants of human AD-MSC proliferation *in vitro* (Han et al., 2014).

In this study, AD-MSCs from donors were prepared as described in registered patents (Kim et al., 2013). The concentration of the SL-13R peptide was determined as previously described (Nii et al., 2021). AD-MSCs were extracted and cultured at 5 x 10^4 cells per plate in DMEM supplemented with or without SL-13R (10 µg/mL) and another novel peptide, KS-13 (10 µg/mL). On days 1, 3, 5, and 7, the expansion of cells was assessed by MTT assay. Here, we demonstrated that both bioactive peptides SL-13R and KS-13 were able to enhance AD-MSC proliferation *in vitro* (Figure 1). As shown in Figure 1A, on day 1, the number of cells in the control, SL-13R-treated, KS-13-treated and genistein-treated samples reached 8214 ± 814, 9475 ± 462 (p=0.07 vs. control), 10770 ± 2307 (p=0.14 vs. control), and 10314 ± 1193 (p=0.06 vs. control), respectively. On day 3, the number of cells in the control, SL-13R-treated, KS-13-treated and genistein-treated samples reached 27310 ± 606, 31680 ± 218 (p=0.0002 vs. control), 32827 ± 1122 (p=0.001 vs. control), and 28463 ± 693 (p=0.09 vs. control), respectively. On day 5, the number of cells in the control, SL-13R-treated, KS-13-treated and genistein-treated samples reached 29853 ± 1272, 29793 ± 691 (p= 0.94 vs. control), 25840 ± 4600 (p=0.21 vs. control), and 22830 ± 845 (p=0.001 vs. control), respectively. On day 7, the number of cells in the control, SL-13R-treated, KS-13-treated and genistein-treated samples reached 15780 ± 690, 21253 ± 240 (p=0.0002 vs. control), 16800 ± 1596 (p=0.36 vs. control), and 9958 ± 1073 (p=0.001 vs. control), respectively. These results demonstrated that both the SL-13R and KS-13 peptides enhanced AD-MSC proliferation compared to that of the control on day 3. Then, the gene expression of MSC stemness markers, including *Nanog*, *Oct3/4*, *Sox2*, *Fgf2*, *Lif,* and *Myc,* and MSC cell surface markers, including CD73, CD105, CD34, CD44, CD146, and HLA-DR in peptide-treated and genistein-treated AD-MSCs on day 3 were examined by RT-qPCR. Table I shows that there were several significant differences in MSC stemness or surface marker gene expression between the peptide– and genistein-treated AD-MSCs.Karyotyping analysis revealed that the cellular numbers of the AD-MSCs were not affected by the peptides SL-13R and KS-13 (Figure 1B). Further investigation is needed to understand the effect of peptide treatment to characteristics of AD-MSCs. These results suggested that the peptides SL-13R and KS-13 maintained the characteristics of AD-MSCs in culture.

The *AHNAK, ANXA2, PLEC,* and *ERLIN2* genes are considered to interact with the SL-13R peptide during HSC expansion (Nii et al., 2021). Therefore, these genes were examined by RT–qPCR in cultured AD-MSCs with or without SL-13R, KS-13; and genistein to determine whether they are involved in AD-MSC expansion.

As shown in Figure 2, the expression of *AHNAK* and *ERLIN2* increased approximately 1.2-fold in SL-13R-treated cells but not in control cells, while the expression of *ANXA2* was significantly increased in KS-13-treated cells compared to control cells. Besides, *PLEC* did not significantly differ between peptide- and genistein-treated cells and control cells. These results suggest that both peptides SL-13R and KS-13 have different functions and mechanisms in AD-MSCs than in HSCs. The statistical significance of differences was determined by two-tailed Student's *t* test and p-value <0.05 was considered statistically significant.

In addition, the aryl hydrocarbon receptor (Ahr) has recently become a promising target for not only immune cell regulation but also stem cell proliferation (Han et al., 2020; Nguyen et al., 2010, Tran et al., 2022). Small molecules such as StemRegenin 1 (SR1), an Ahr antagonist, induce *ex vivo* expansion of $CD34^+$ cells

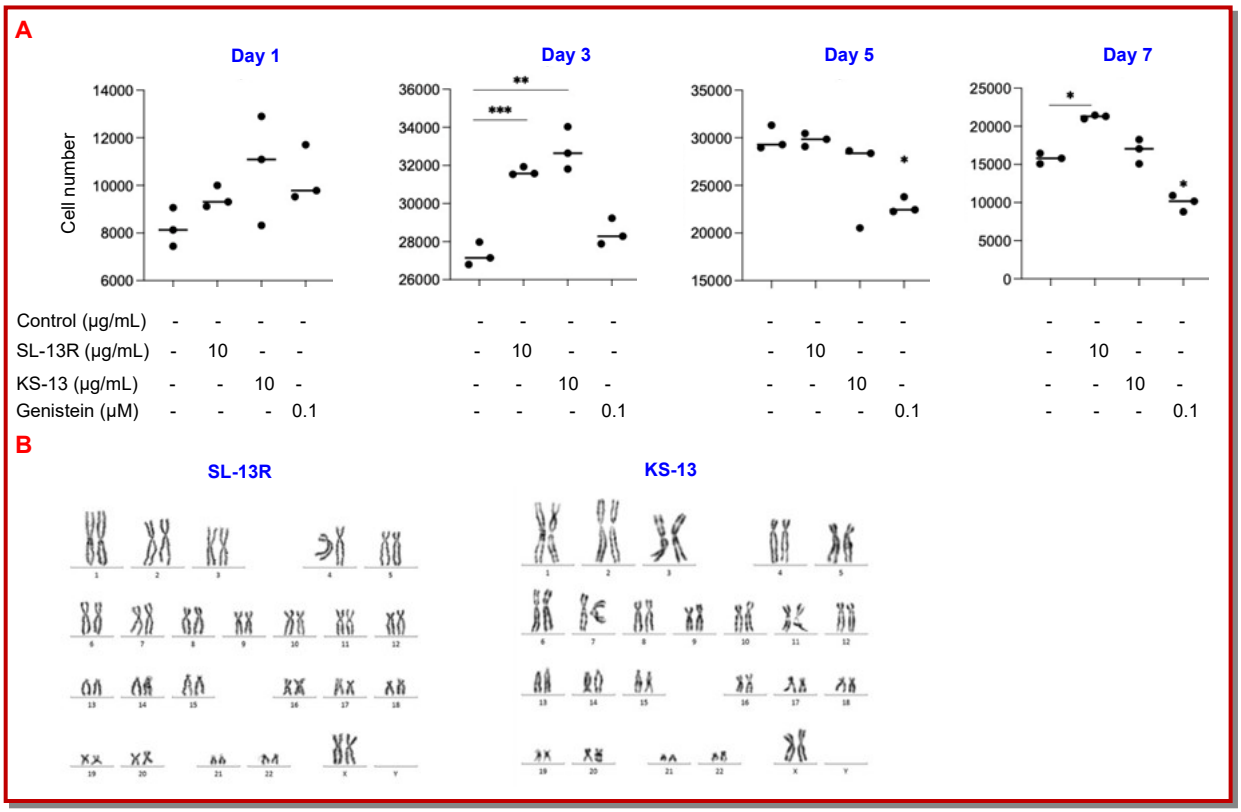

Figure 1: The expansion of human AD-MSCs by SL-13R, KS-13 and genistein *in vitro*. **A**) The number of AD-MSCs after days 1, 3, 5, and 7 in the control, SL-13R-treated, KS-13-treated and genistein-treated samples was examined by MTT. **B)** After day 2, peptide-treated AD-MSCs were harvested for karyotyping analysis (n=3). The results are the representative of three independent experiments

Table I

Expression of *different parameters after 3 days*

	Genistein	SL-13R	KS-13
Nanog[a]	1.2 ± 0.3	0.7 ± 0.1 (p=0.046 vs. KS-13)	1.0 ± 0.2
Oct3/4[a]	1.0 ± 0.1	0.8 ± 0.2	0.9 ± 0.1
Sox2[a]	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
Lif[a]	0.8 ± 0.0	0.9 ± 0.3	1.4 ± 0.3 (p=0.038 vs. Genistein)
Fgf2[a]	0.8 ± 0.1	1.1 ± 0.1 (p=0.021 vs. Genistein)	1.0 ± 0.1 (p=0.037 vs. Genistein)
Myc[a]	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
CD73[a]	1.1 ± 0.3	0.8 ± 0.1	1.0 ± 0.3
CD105[a]	1.1 ± 0.1	1.0 ± 0.3	1.1 ± 0.3
CD34[a]	1.0 ± 0.1	1.3 ± 0.5	1.0 ± 0.1
CD44[a]	0.9 ± 0.2	0.8 ± 0.1	1.1 ± 0.2
CD146[a]	0.7 ± 0.1	0.8 ± 0.3	1.0 ± 0.3
HLA-DR[a]	0.9 ± 0.5	0.6 ± 0.4 (p=0.009 vs. KS-13)	1.7 ± 0.1

[a]When compared to control (GAPDH); Concentration of genistein (0.1 μM), SL-13R (10 μg/mL), KS-13 (10 μg/mL) were used; Data are mean ± S.D.

from primary human HSCs by activating Ahr (Boitano et al., 2010). Furthermore, it was recently demonstrated that SR1 induces the expansion of $CD34^+$ cells (Nii et al., 2021). This preliminary data showed that Ahr ligands such as 6-formylindolo[3,2-b]carbazole (FICZ) and kynurenic acid (KA) can significantly induce the

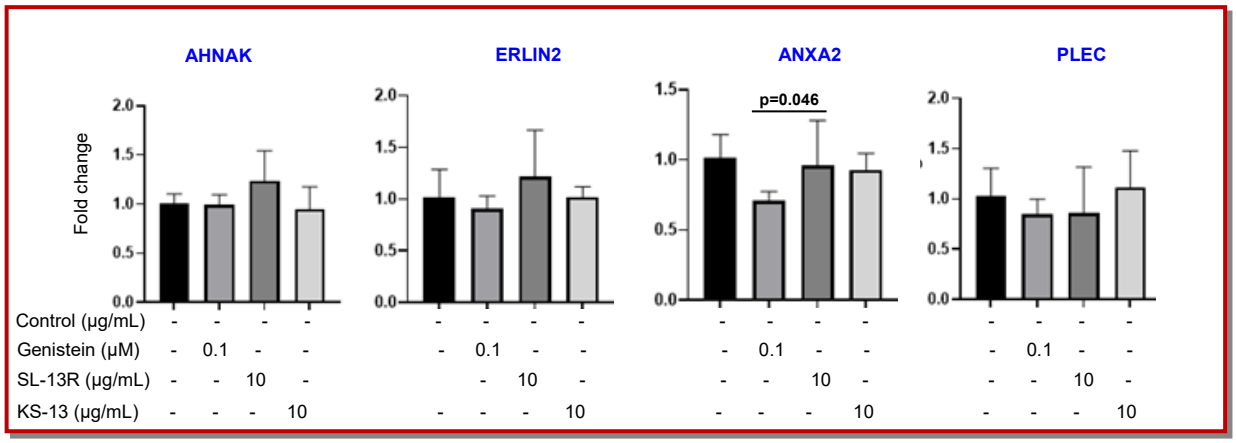

Figure 2: Gene expression of *AHNAK, ANXA2, PLEC and ERLIN2* with or without SL-13R, KS-13 and genistein in cultured AD-MSCs after day 3. The gene expression of *AHNAK, ANXA2, PLEC* and *ERLIN2* in cultured AD-MSCs was measured by qPCR (n=3). The results are the representative of three independent experiments. Data are mean ± SD

expansion of AD-MSCs (unpublished data). Whether the bioactive peptides SL-13R and KS-13 regulate the functions of Ahr that promotes AD-MSC expansion *in vitro* is under investigation. Therefore, the use of bioactive peptides, natural compounds, and small molecules that interact with Ahr may potently improve the efficacy of AD-MSC-based therapies.

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Ethical Issue: The study was performed in accordance with protocols approved by the ethnics committee of the Hanoi Obstetrics and Gynecology Hospital [Ref. No. 2206 CN/PS]

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

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