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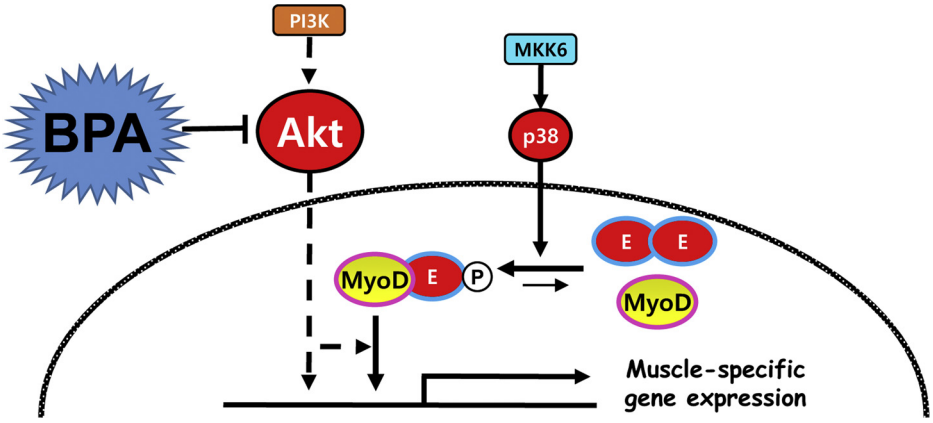
Bisphenol A and estradiol impede myoblast differentiation through down-regulating Akt signaling pathway

Ga-Yeon Go[a,1], Sang-Jin Lee[a,1], Ayoung Jo[a], Jae-Rin Lee[b], Jong-Sun Kang[b], Mihi Yang[a,*], Gyu-Un Bae[a,*]

[a] Research Center for Cell Fate Control, College of Pharmacy, Sookmyung Women's University, Seoul 04310, Republic of Korea
[b] Department of Molecular Cell Biology, Single Cell Network Research Center, Sungkyunkwan University School of Medicine, Suwon 16419, Republic of Korea

GRAPHICAL ABSTRACT



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ABSTRACT

Bisphenol A (BPA), one of the most widespread endocrine disrupting chemicals, is known as an artificial estrogen, which interacts with estrogen receptor (ER). In this study, we investigated the effects of BPA and estradiol on myoblast differentiation and the underlying signaling mechanism. Exposure to BPA (0.01–1 μM) in mouse myoblast C2C12 cells attenuated myogenic differentiation via the reduced expression of muscle-specific genes, such as myosin heavy chain (*MHC*), *MyoD,* and *Myogenin,* without the alteration of cell proliferation and viability. BPA-exposed C2C12 myoblasts also showed a reduction of Akt phosphorylation ((37–61) %, $p < 0.001$), a key event for myogenesis. Similarly to BPA, estradiol (0.01–1 μM) reduced the expression of muscle-specific proteins and the formation of multinucleated myotubes, and attenuated the muscle differentiation-specific phosphorylation of Akt ((42–59) %, $p < 0.001$). We conclude that BPA and estradiol suppress myogenic differentiation through the inhibition of *Akt* signaling.

* Corresponding authors at: Research Center for Cell Fate Control, College of Pharmacy, Sookmyung Women's University, Cheongpa – ro 47 – gil 100, Yongsan – Gu, Seoul 04310, Republic of Korea.
E-mail addresses: myang@sookmyung.ac.kr (M. Yang), gbae@sookmyung.ac.kr (G.-U. Bae).
[1] These authors contributed equally to this work.

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1. Introduction

Estrogenic endocrine disrupting chemicals (EDCs) are a structurally diverse group of compounds that either mimic or antagonize the effect of endogenous estrogens (Tyler et al., 1998). The biological activities of estrogens are mediated by two isoforms of estrogen receptors (ERs), namely ERα and ERβ, which are members of the nuclear receptor superfamily of ligand-mediated transcriptional factors (Heldring et al., 2007). Recently, ERα and ERβ have been identified in myoblasts and skeletal muscles (Milanesi et al., 2008; Wiik et al., 2009). Therefore, estrogens seem to act on skeletal muscle through ER isoforms (Ogawa et al., 2011).

As one of the most commonly produced synthetic chemicals worldwide, bisphenol A [BPA, 4,4′-(propane-2,2-diyl) diphenol], which is known as an endocrine disruptor, has been extensively used in polycarbonate plastics and epoxy resins that are found in a wide range of consumer products, such as food containers, food cans, water bottles, baby bottles, dental sealants, and water pipes. However, it is released after exposure to elevated temperature (Swedenborg et al., 2009; Yan et al., 2011). BPA mimics the natural hormone estrogen and binds to estrogen receptors (ERs), which causes negative effects on health (Lee et al., 2018; Li et al., 2015; Rubin et al., 2001; Swedenborg et al., 2009; Yang et al., 2009). For example, BPA showed a wide-range of physiological toxicities, including anti-thyroid hormone effects or non-classical targeting, such as bones, cardiovascular tissue, pancreas, adipose tissue, and the immune system (Richter et al., 2007; Rubin et al., 2001; Vandenberg et al., 2010). In particular, a recent study showed BPA induces cardiac fibrosis by activating the ERK1/2 pathway (Hu et al., 2016). Although these BPA toxicities are estimated via the activation of intracellular signaling pathways associated with ERs (Babiker et al., 2002), the toxic mechanisms of BPA have so far remained unclear, particularly in skeletal muscle.

Differentiation of skeletal myoblasts is a tightly orchestrated process that involves myoblast proliferation, cell cycle withdrawal, expression

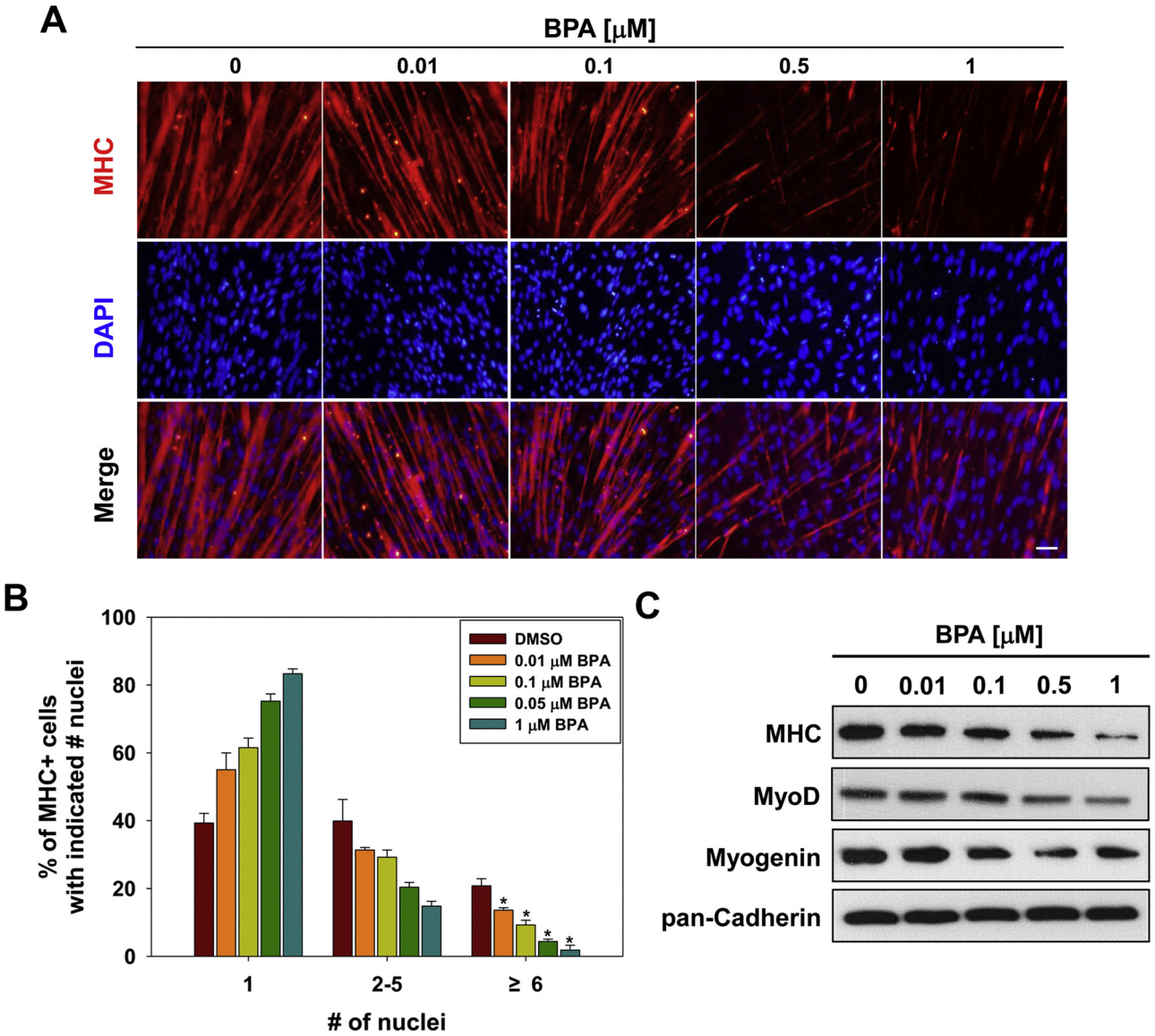

Fig. 1. BPA inhibits myogenic differentiation. (A) C2C12 myoblasts were treated with the indicated concentration of BPA, and induced to differentiate in DM **for 2 days**, followed by immunostaining for MHC expression (red) and DAPI to visualize nuclei (blue) to reveal myotube formation. Scale bar, 100 μm. (B) Quantification of myotube formation from data shown in panel (A). Values represent the means of triplicate determinations ± 1 SD. The experiment was repeated three times, with similar results. Asterisks indicate significant difference from the control. $^{*}P < 0.01$. (C) Cell lysates from similar experiments shown in panel (A) were subjected to immunoblotting with antibodies to MHC, MyoD, Myogenin, and pan-Cadherin as a loading control. The experiment was repeated three times, with similar results. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)