세포 노화억제와 젊은 세포로의 재생까지 가능한 새로운 노화원인물질 발견

• 교신저자 : 장익순(바이오융합) / 여의주(가천대)교수

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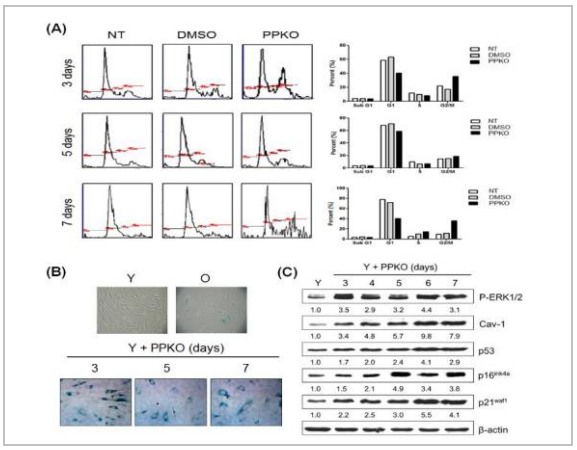
연구내용

첨단연구장비인 기체크로마토그래피 질량분석시스템 (Gas Chromatography-Mass Analysis System/ GC-Ms)을 활용하여 늙은 섬유아세포를 둘러싸고 있는 세포외 기질(Extracellular Matrix/ECM)에서 세포 노화를 촉진하는 소형 화합물인 PPKO(Phenyl2-pyridyl Ketoxime)을 발견함.
이러한 발견은 젊은 세포와 노화 세포를 배양한 후 배양접시의 표면에 있는 세포외기질을 획득하고 이를 GC-MS를 통해 분석한 후, 차이나는 물질들을 비교분석하는 과정을 통해 이루어졌음.
또한, 늙은 세포에서 PPKO에 의한 활성산소와 산화질소 생성을 막을 경우 젊은 세포로 기능회복 되는 것을 확인하였음.

[그림 1] 기체크로마토그래피질량분석시스템(GC-MS)



[그림 2] PPKO에의한G2/M 세포주기정체(A), SA-β-gal 염색증가(B), 노화관련단백질의발현증가(C)

기대효과

이번 성과는 노화를 억제하거나 유발시킬 수 있고 나아가 노화된 세포를 다시 젊게 만들 수 있는 세포 노화 제어 연구에 응용될 수 있음.

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Phenyl 2-pyridyl ketoxime induces cellular senescence-like alterations via nitric oxide production in human diploid fibroblasts

Kyeong Eun Yang,[†] Hyun-Jin Jang,[†] In-Hu Hwang,[2] Young-Ho Chung,[1] Jong-Soon Choi,[1] Tae-Hoon Lee,[3] Yun-Jo Chung,[4] Min-Seung Lee,[5] Mi Young Lee,[6] Eui-Ju Yeo[3] and Ik-Soon Jang[1]

[1]Drug & Disease Target Group, Division of Bioconvergence Analysis, Korea Basic Science Institute, Daejeon 305-333, Korea
[2]Department of Physiology, Korea University College of Medicine, Seoul 02841, Korea
[3]Department of Oral Biochemistry, Dental Science Research Institute, Chonnam National University, Gwangju 500-757, Korea
[4]Center for University-Wide Research Facilities, Chonbuk National University, Jeonju, Korea
[5]Department of Biochemistry, College of Medicine, Gachon University, Inchon 406-799, Korea
[6]ICM Convergence Research Division, Korea Institute of Oriental Medicine, Daejeon 305-811, Korea

Summary

Phenyl-2-pyridyl ketoxime (PPKO) was found to be one of the small molecules enriched in the extracellular matrix of near-senescent human diploid fibroblasts (HDFs). Treatment of young HDFs with PPKO reduced the viability of young HDFs in a dose- and time-dependent manner and resulted in senescence-associated β-galactosidase (SA-β-gal) staining and G2/M cell cycle arrest. In addition, the levels of some senescence-associated proteins, such as phosphorylated ERK1/2, caveolin-1, p53, $p16^{INK4a}$, and $p21^{waf1}$, were elevated in PPKO-treated cells. To monitor the effect of PPKO on cell stress responses, reactive oxygen species (ROS) production was examined by flow cytometry. After PPKO treatment, ROS levels transiently increased at 30 min but then returned to baseline at 60 min. The levels of some antioxidant enzymes, such as catalase, peroxiredoxin II and glutathione peroxidase I, were transiently induced by PPKO treatment. SOD II levels increased gradually, whereas the SOD I and III levels were biphasic during the experimental periods after PPKO treatment. Cellular senescence induced by PPKO was suppressed by chemical antioxidants, such as N-acetylcysteine, 2,2,6,6-tetramethylpiperidinyloxy, and L-buthionine-(S,R)-sulfoximine. Furthermore, PPKO increased nitric oxide (NO) production via inducible NO synthase (iNOS) in HDFs. In the presence of NOS inhibitors, such as L-NG-nitroarginine methyl ester and L-NG-monomethylarginine, PPKO-induced transient NO production and SA-β-gal staining were abrogated. Taken together, these results suggest that PPKO induces cellular senescence in association with transient ROS and NO production and the subsequent induction of senescence-associated proteins.

Key words: cellular senescence; human diploid fibroblast; nitric oxide; phenyl 2-pyridyl ketoxime; reactive oxygen species.

Correspondence
Ik-Soon Jang, Drug & Disease Target Group, Division of Bioconvergence Analysis, Korea Basic Science Institute, Gwahangno 113, Yuseong-gu Daejeon 305-333, Korea. Tel.: +82 (0)42 865 3430; fax: +82 (0)42 865 3419; e-mail: jangikim@kbsi.re.kr
and
Eui-Ju Yeo, Department of Biochemistry, College of Medicine, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 406-799, Korea. Tel.: +82 32 820 4742; fax: +82 32 820 4744; e-mail: eujuy@gachon.ac.kr
[†]These authors contributed equally to this work.

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Abbreviations

BSO	L-buthionine-(S,R)-sulfoximine
BCA	bicinchoninic acid
DCF-DA	2′,7′-dichlorofluorescein diacetate
DMEM	Dulbecco's modified Eagle's medium
ECM	extracellular matrix
ECL	enhanced chemiluminescence
FBS	fetal bovine serum
GPX	glutathione peroxidase
HDFs	human diploid fibroblasts
L-NAME	L-NG-nitroarginine methyl ester
L-NMMA	L-NG-monomethylarginine
MTT	3-(4,5-dimethylthiazol-e-yl)-2,5-diphenyltetrazolium bromide
NAC	N-acetylcysteine
NO	nitric oxide
NOS	nitric oxide synthase
PI	propidium iodide
P-ERK	phosphorylated extracellular signal-regulated kinase
Prdx	peroxiredoxin
PD	population doubling
PI	propidium iodide
PPKO	phenyl 2-pyridyl ketoxime
ROS	reactive oxygen species
SA-β-gal	senescence-associated β-galactosidase
SOD	superoxide dismutase
TEMPO	2,2,6,6-tetramethylpiperidinyloxy
X-gal	5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside

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