

[4/18/2008(Fri) 11:30~14:05/1st FL]

Regulation of Estrogen Receptor Under Hypoxia in Breast Cancer Cells

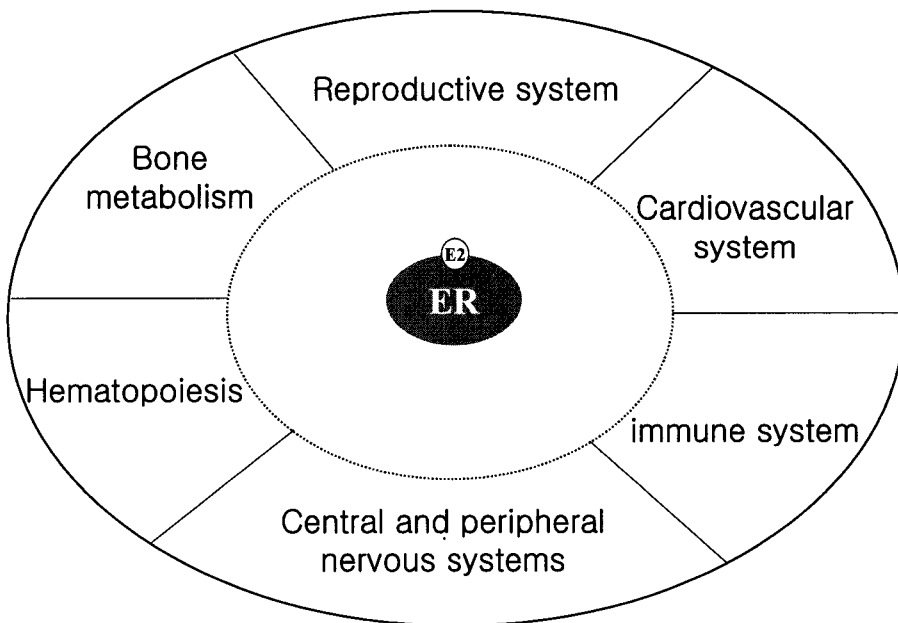
Youngjoo Lee

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Biotechnology, Sejong University, Seoul, Korea.

Previously, we have shown that hypoxia, through HIF-1, induces ligand-independent ER α activation and the physical interaction of HIF-1 and ER α . However, the effect of hypoxia on the transactivation of ER β is not yet known. In the present study, we found that hypoxia activated the ER β -mediated transcriptional response in the HEK 293 cell line, as determined by the transient expression of ER β and ER-responsive reporter plasmids. The hypoxia-induced estrogen response element-mediated transcriptional response was dependent on ER β expression and was inhibited by the ER antagonist ICI 182,780. Transactivation of ER β was induced by the expression of HIF-1 α under normoxic conditions, as determined by the expression of oxygen-independent stable GFP-HIF-1 α . HIF-1 α -induced ER β transactivation was abolished by the inhibition of HIF-1 α activation. This was determined by using chemical inhibitors for the MAPK pathway. In addition, HIF-1 α interacted with ER β in a mammalian-two hybrid assay. We conclude that hypoxia activates ER β in a ligand-independent manner, possibly through the interaction of HIF-1 α and ER β .

Modulation of Actions of Estrogen Receptors under Hypoxia

ER plays an important role in various systems



Structural domains of the human ER



Transcriptional Activation

AF1

AF2

Nuclear Localization

Dimerization

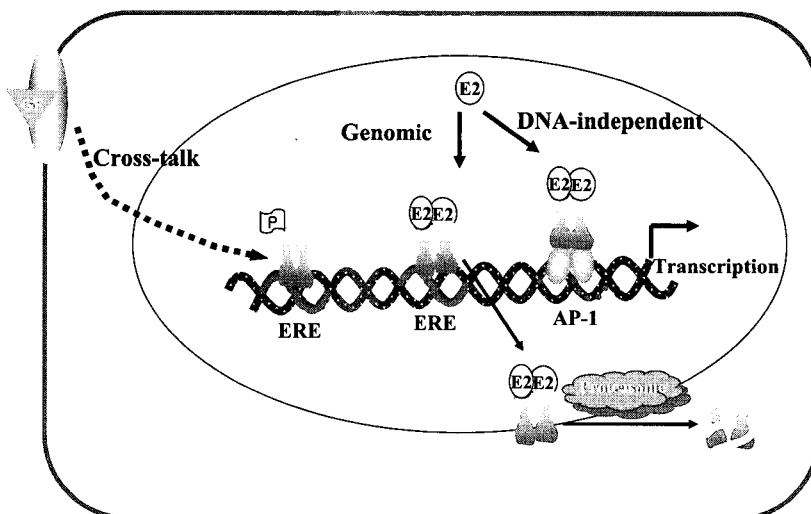
DNA-Binding

Co-Activator Binding

Co-Repressor Binding

Steroids 2000:65:227

Estrogen receptor signaling



Modulation of unliganded ER signaling by various factors

- Leptin

Catalano S et. al., Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells, *J Biol Chem* 2004

- Dioxin

Fumiaki Ohtake et. al., Modulation of estrogen receptor signaling by association with the activated dioxin receptor, *Nature* 2003

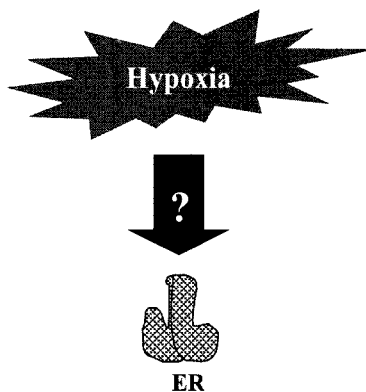
- Oxytosine

Cassoni P et. al., Oxytocin modulates estrogen receptor alpha expression and function in MCF7 human breast cancer cells. *Int J Oncol* 2002

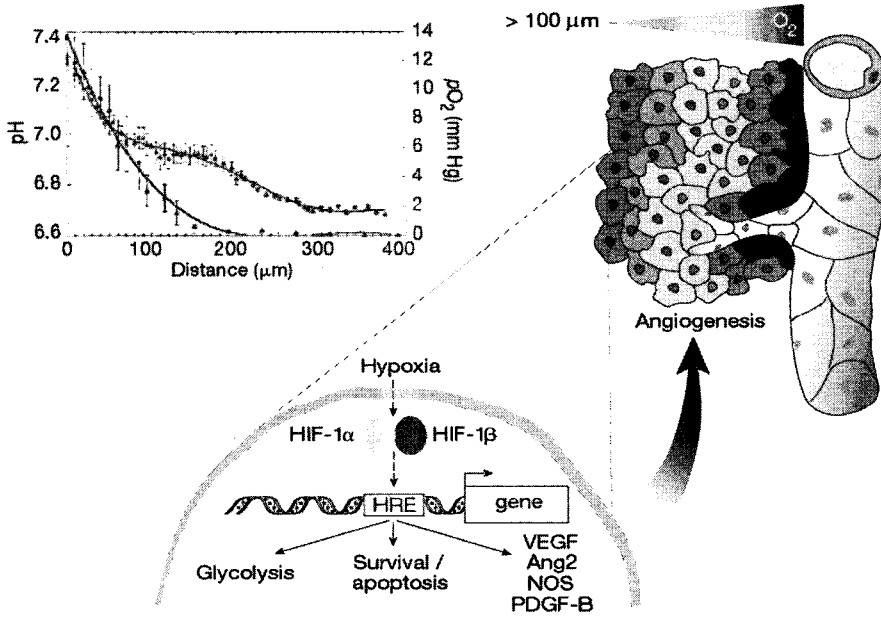
- Hypoxia

Stoner M et. al., Hypoxia induces proteasome-dependent degradation of estrogen receptor alpha in ZR-75 breast cancer cells, *Mol Endocrinol* 2002

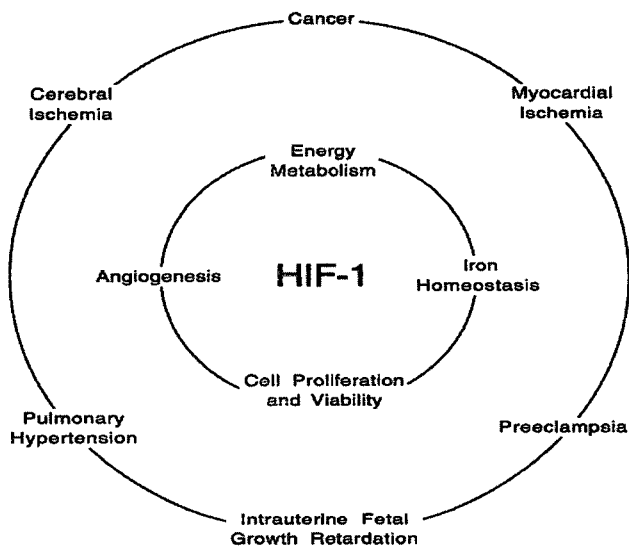
What mechanism of regulation of ER under hypoxia?



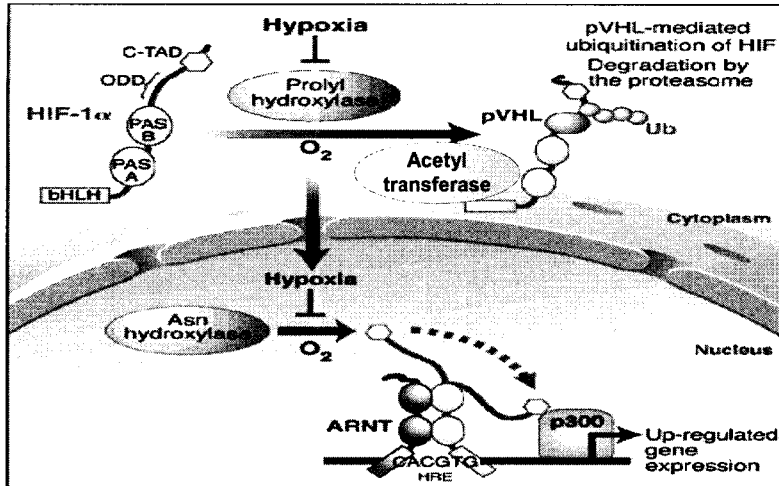
Role of hypoxia in tumor angiogenesis



HIF-1 in key physiological and pathological process

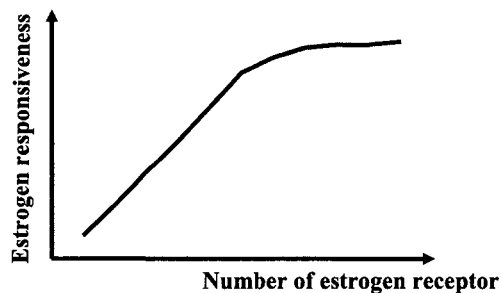


Regulation of the HIF-1 expression by oxygen level (through hydroxylation, acetylation)



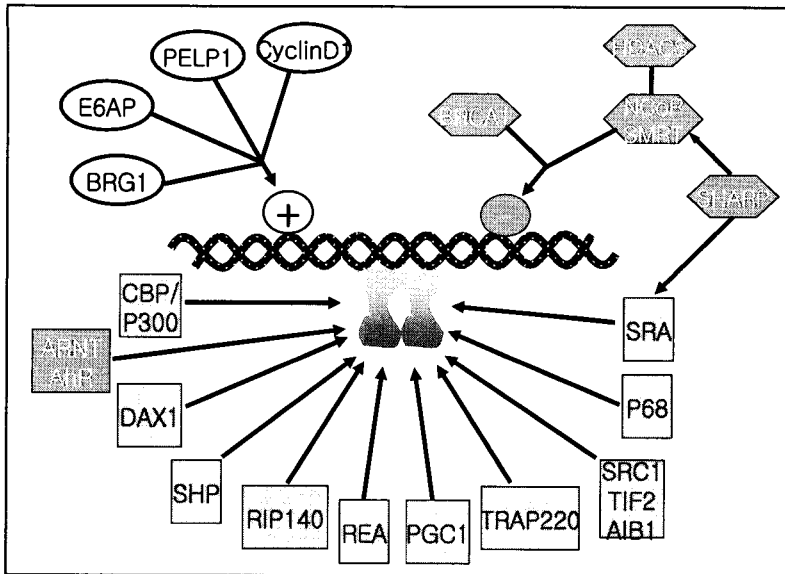
Importance of regulation of ER levels

Sensitivity of cells to estrogen is depend on the number of ERs



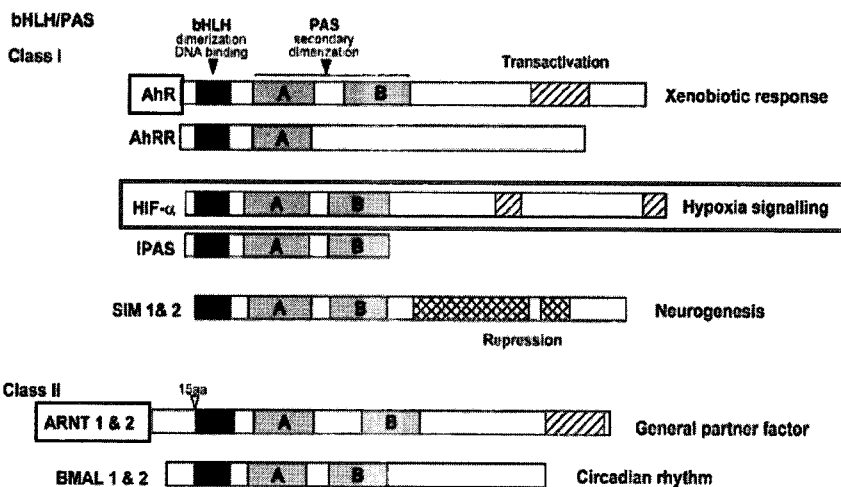
- Breast cancer : high levels of ER α and high sensitivity to estrogen
- Anti-estrogen ICI : repression of response of E2 through rapid degradation of ER α a protein

Coregulators & Activation of ER



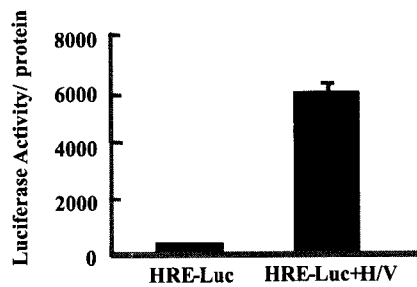
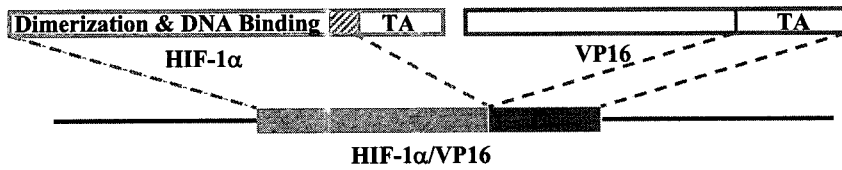
Adapted from *Science* 2002;296:1642

bHLH transcription factor family members

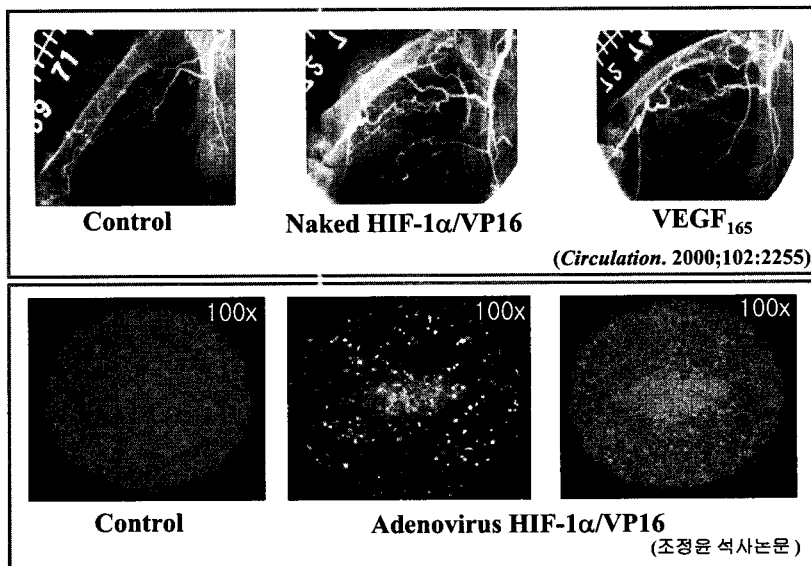


Int J Biochem Cell Biol 2004;36:189

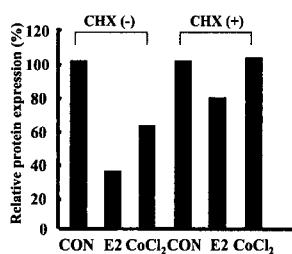
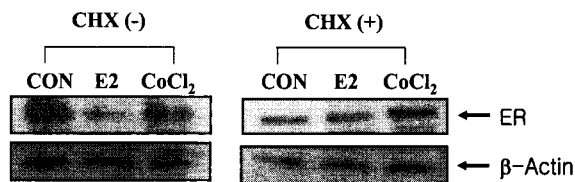
Schematic and transactivation of HIF-1 α /VP16 hybrid



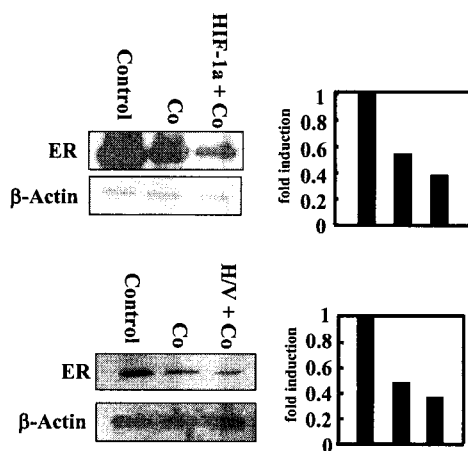
In vivo function of HIF-1 α /VP16 and adenovirus HIF-1 α /VP16 plaque on 293 cells



ER α downregulation by hypoxia mimic agent CoCl₂ ; role of protein synthesis

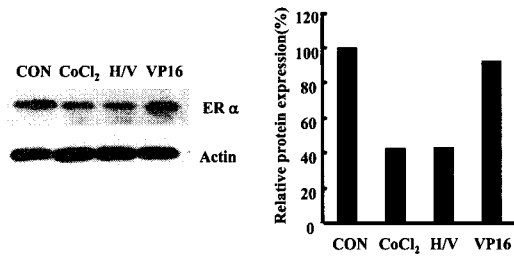


ER α downregulation by hypoxia mimic agent CoCl₂ ; effect of HIF-1 α overexpression

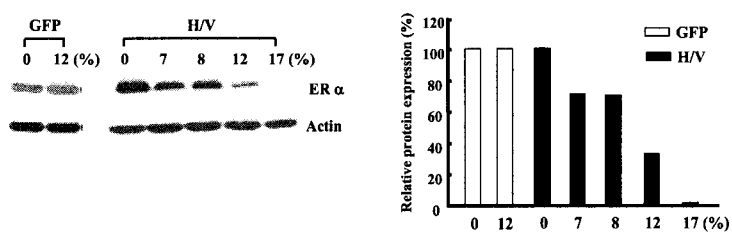


Effects of HIF-1 α /VP16 hybrid on ER α protein

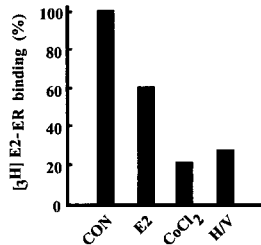
A



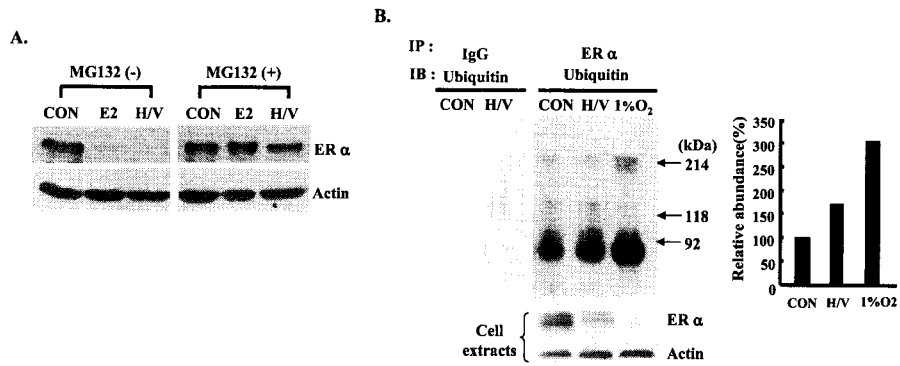
B



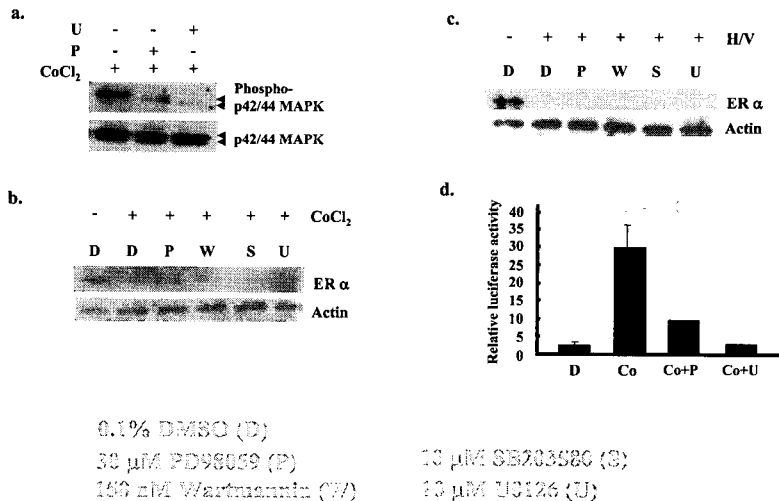
C



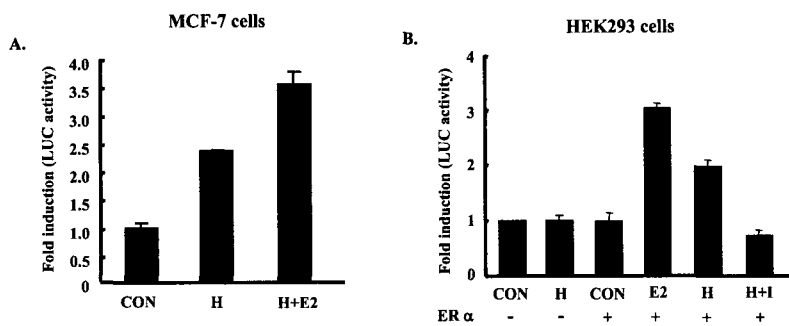
Effects of proteasome inhibitor on HIF-1 α /VP16-induced degradation of ER α protein



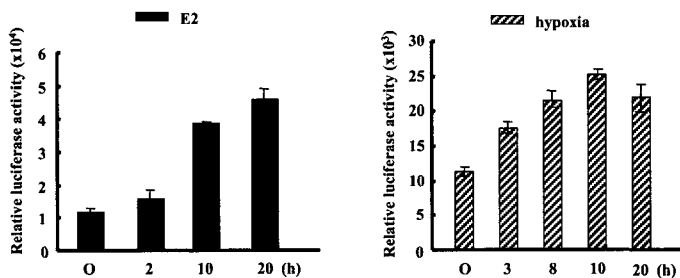
Effects of protein kinase inhibitors on ER α downregulation



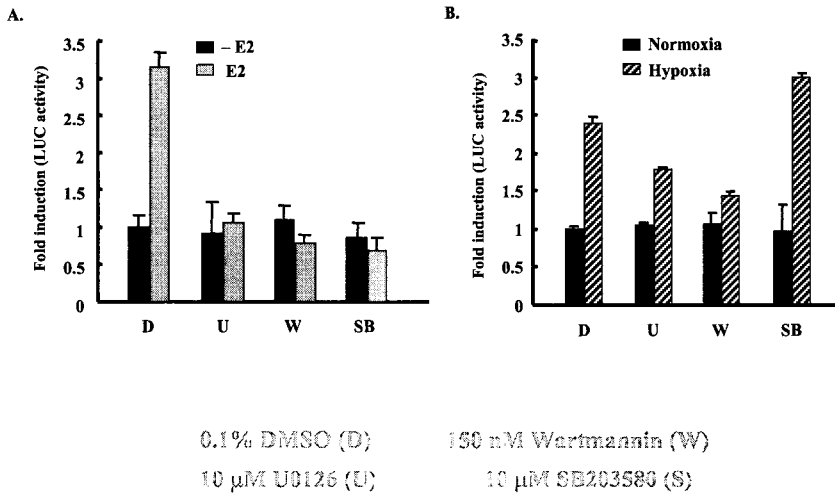
Effect of hypoxia on ER α -mediated transactivation



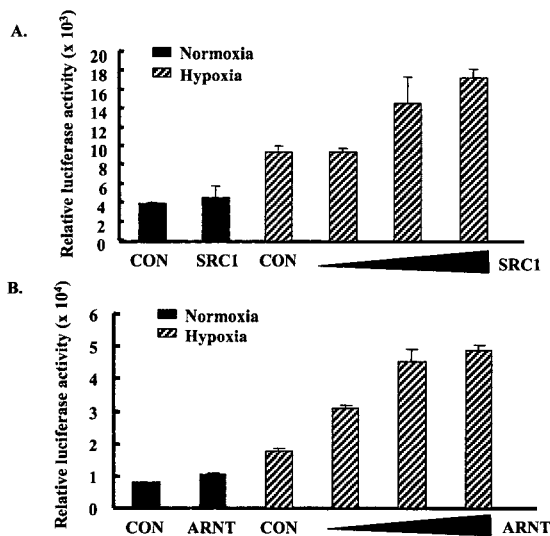
Time course of estrogen or hypoxia induced ER α transactivation



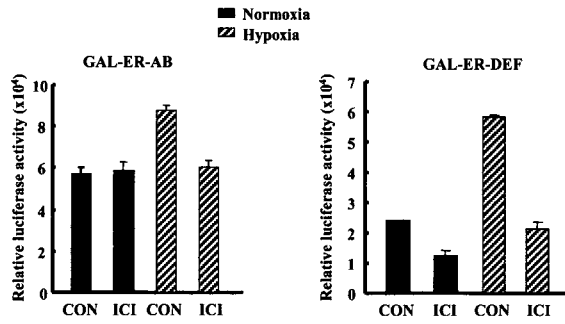
Effect of kinase inhibitors on hypoxia-induced ER α transactivation



Effect of SRC1 or ARNT overexpression on hypoxia-induced ER α transactivation

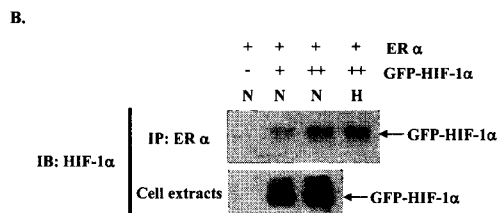
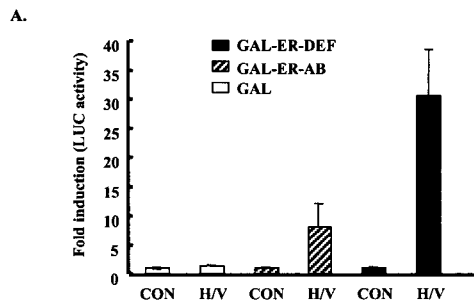


Effects of hypoxia on AF-1 or AF-2 activation domain of ER α

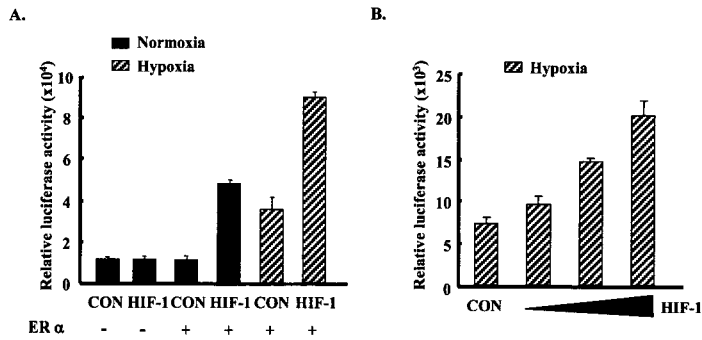


*. B region contains an AF-1; E region contains an AF-2

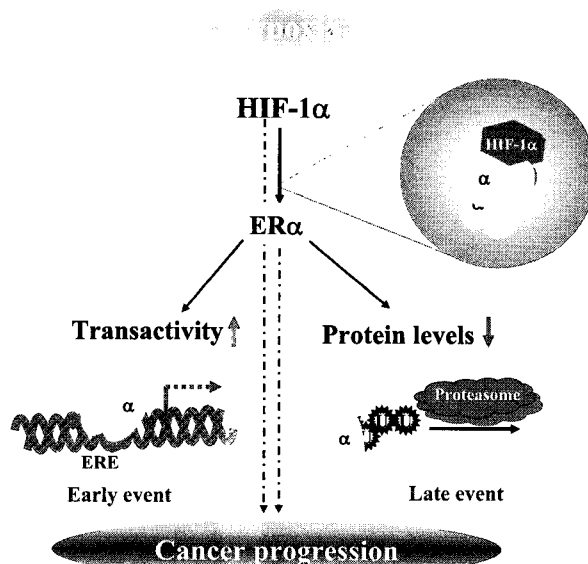
Interaction of ER α and HIF-1 α



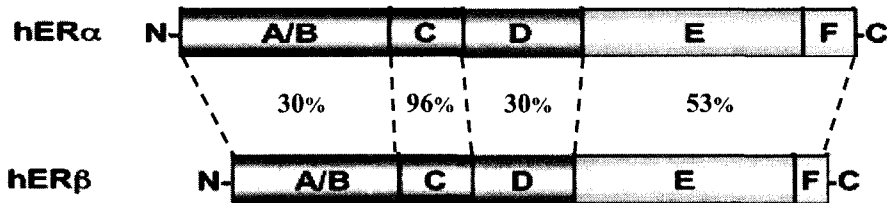
Effect of HIF-1 on ER α -mediated transactivation



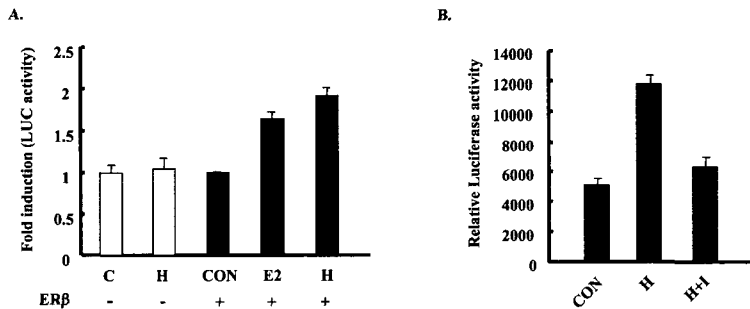
Modulation of ER α under hypoxia; role of HIF-1 α



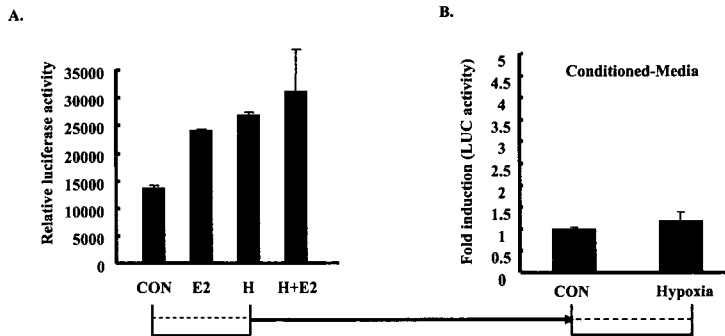
Identities between corresponding regions of human ER α and ER β



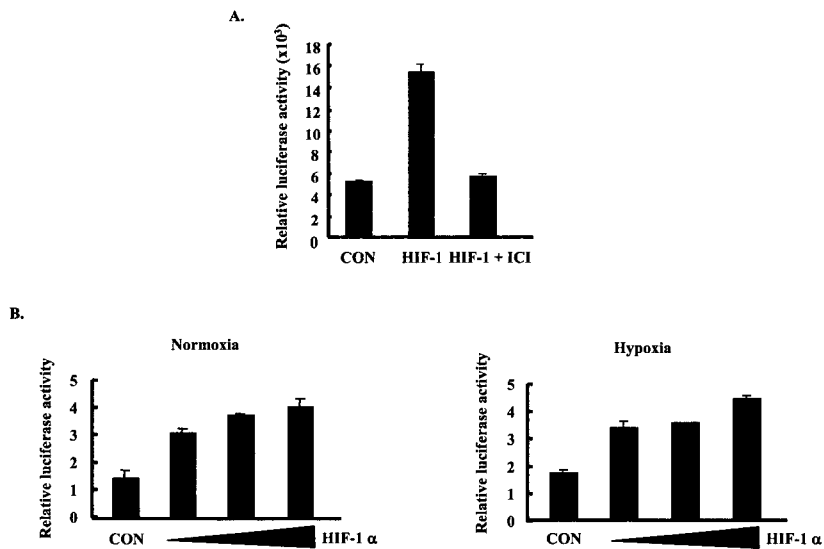
Effect of hypoxia on ER β -mediated transactivation



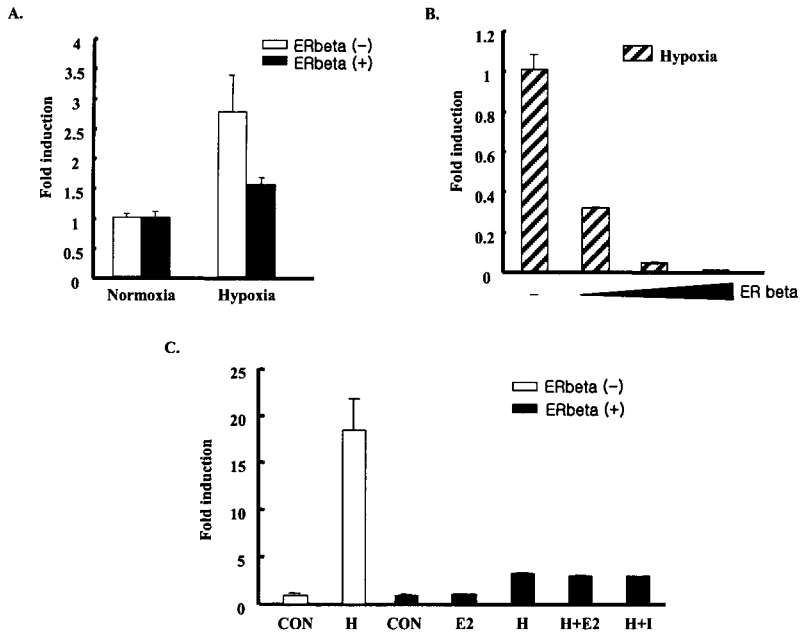
Effect of hypoxia on ER β -mediated transactivation



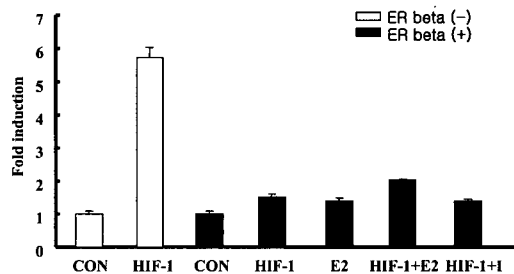
Effect of HIF-1 on ER β -mediated transactivation



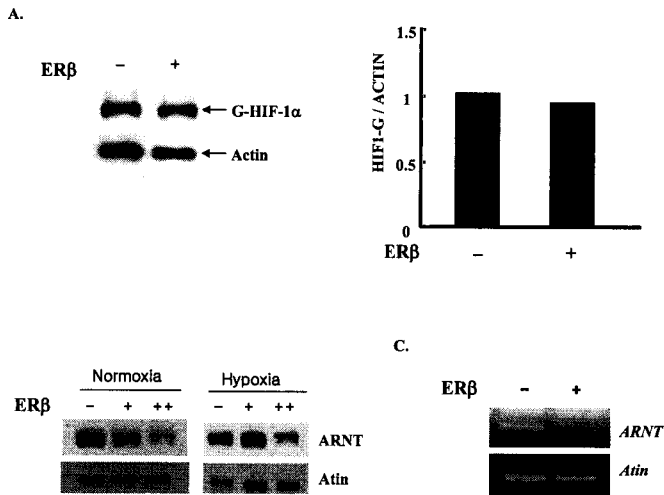
Effect of ER β on HRE-mediated transcription



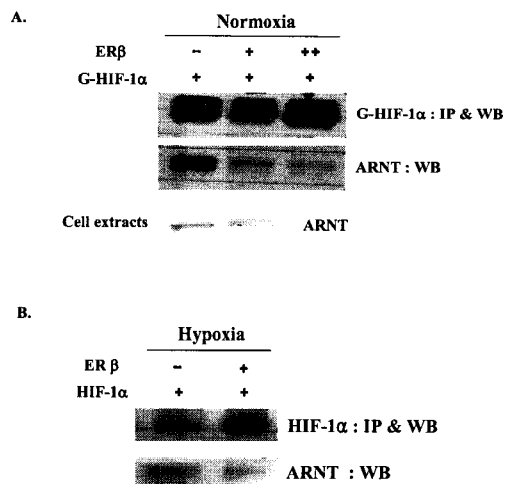
Effect of ER β on HIF-1-mediated transcription



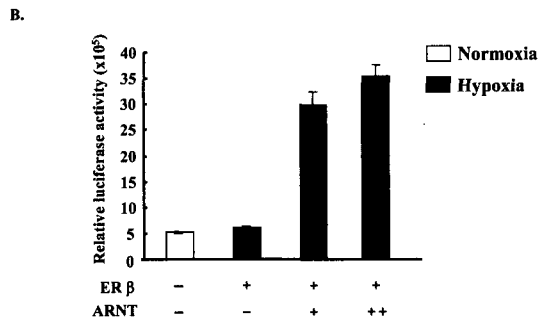
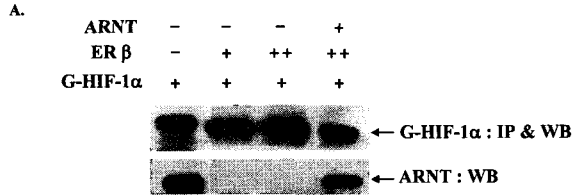
Effect of ER β on HIF-1 α or ARNT levels



Effect of ER β on HIF-1 α /ARNT dimerization levels



Effect of ARNT overexpression on transrepression of HIF-1 induced by ER β



Effect of ER β on hypoxia inducible gene; EPO RNA level

