

Molecular Mechanisms Underlying PGF2 α -induced Hypertrophy of Vascular Smooth Muscle Cells

Chunyuan FAN,^{*,a} Masato KATSUYAMA,^b Haiyan WEI,^c Qingjie XIA,^a
Wentao LIU,^a and Chihiro YABE-NISHIMURA^b

^aDepartment of Nephrology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, 1# Keyuan Road 4, Gaopeng Street, High Technological Development Zone, Chengdu, 610041, China, ^bDepartment of Pharmacology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan, and ^cState Key Laboratory of Biotherapy, West China Hospital and School of Life Science, Sichuan University, 1# Keyuan Road 4, Gaopeng Street, High Technological Development Zone, Chengdu, 610041, China

(Received September 14, 2009; Accepted October 23, 2009)

The present review focuses primarily on the studies we made in recent years to improve the understanding of the molecular mechanisms of PGF2 α -induced hypertrophy of Vascular Smooth Muscle Cells (VSMC). In this review, we will summarize the recent findings in the context of the PGF2 α signaling pathway in three parts: PGF2 α binding to its receptor, transactivation of EGF receptor, two independent signaling transduction pathways increasing the expression of NOX1 gene.

Key words—PGF2 α ; NOX1; hypertrophy; Vascular Smooth Muscle Cells (VSMC)

INTRODUCTION

Reactive oxygen species (ROS) including superoxide (O²⁻) and hydrogen peroxide (H₂O₂) are recognized as important signaling molecules in cardiovascular tissues. It has been shown that nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidases are the major source of O²⁻ in vascular cells and myocytes,¹⁻³ and their activities are increased by the vasoactive factors such as angiotensin II and thrombin.⁴⁻⁶ Prostaglandin F₂ α (PGF2 α) is a vasoactive factor that leads to constriction and hypertrophy of vascular smooth muscle cells (VSMC) and cardiac myocytes.⁷⁻⁹ Although O²⁻ produced by NADPH oxidase is involved in the PGF2 α -induced hypertrophy of VSMC, the signaling pathways mediating this effect remains unknown.¹⁰⁻¹² To clarify the molecular mechanisms underlying PGF2 α -induced hypertrophy of VSMC, we make research on a rat vascular smooth muscle cell line, A7r5.

The present review focuses primarily on the studies we made in recent years to improve the understanding of the molecular mechanisms of PGF2 α -induced hypertrophy of VSMC. In brief, as illustrated in Fig. 1, PGF2 α exerts its biological actions through binding to its specific receptor, FP, on plasma membranes.

Upon ligand binding, the FP signals to downstream molecules, protein kinase C δ (PKC δ). And then the activation of Src and MMPs, leading to the excision of heparin-binding EGF, is involved in the transactivation of the EGF receptor by PGF2 α . Once EGF receptor is phosphorylated, there exist two independent pathways, PI3K-ATF1-MEF2B and MEK-ERK1/2. Activating transcription factor 1 (ATF-1), as a PI3K intracellular substrate, is activated. Activated ATF-1 are translocated into the nucleus to regulate NOX1 gene expression *via* myocyte enhancer factor 2B (MEF2B) binding to the promoter region of rat NOX1 gene. Furthermore, we also identified the other signaling pathway including extracellular signal-regulated kinases1/2 (ERKs1/2), which as the downstream molecule of MEK regulates positively the expression of NOX1 gene. In this review, we will discuss the recent findings in the context of the PGF2 α signaling pathway in three parts: PGF2 α binding to its receptor, transactivation of EGF receptor, two independent signaling transduction pathways increasing the expression of NOX1 gene.

Firstly, upon Interacting with Its Receptor, PGF2 α Increases the O²⁻ Generation through Upregulating the Expression of NOX1

The phagocyte NADPH oxidase constitutes two plasma membrane-spanning subunits, gp91phox

*e-mail: fanchunyuan38@yahoo.com.cn

(NOX2) and p22phox; two cytosolic subunits, p47phox and p67phox; and a small G protein, Rac. To date, seven homologs of the catalytic subunit including NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2 have been reported.¹³⁻¹⁵⁾ Among the NOX superfamily, NOX1, NOX4 and NOX5 have been detected in VSMC.¹⁵⁻¹⁷⁾ In our studies, no alteration in the expression levels of NOX4 was found in A7r5 cells stimulated with PGF2 α . As stimulation of FP causes a cytosolic mobilization of Ca²⁺, NOX5, which produces O²⁻ in a Ca²⁺-dependent manner, could be activated indirectly by PGF2 α . But NOX5 has not been identified in rodents and we could not detect the expression of NOX5 homolog in A7r5 cells by RT-PCR with primers designed from the reported human sequence.¹⁵⁾ In addition, stimulation with a calcium ionophore, A23187 did not affect the level of O²⁻ in the PGF2 α treated cells. Accordingly, the contribution of other members of the NOX family to the PGF2 α -induced generation of O²⁻ demonstrated in A7r5 cells seems to be minimal. As a result, NOX1/NADPH oxidase-derived O²⁻ plays pivotal roles in PGF2 α -mediated hypertrophy of VSMC.^{18,20,23,25-27)}

The induction of NOX1 expression by PGF2 α seems to be mediated specifically by its receptor, FP, not by other prostanoid receptors. There are several possible reasons for this. First, (+)-fluprostenol, which upregulated NOX1 mRNA in a dose-dependent manner, is a highly selective FP agonist. It was reported that this compound does not react with other prostanoid receptors.¹⁹⁾ Second, expression of FP was detected in A7r5 cells,⁸⁾ and we also verified its expression by RT-PCR. Third, the concentration of PGF2 α that elicited NOX1 expression was 10⁻¹⁰ M, much lower than those of other vasoactive prostanoids investigated.

Subsequently, PKC δ Mediates Transactivation of the EGF Receptor through Src, MMP

A time-dependent increase in the phosphorylation of the EGF receptor was demonstrated in the cells stimulated with PGF2 α , while tyrphostin AG1478, an inhibitor of EGF receptor, significantly suppressed PGF2 α -induced increase in NOX1 mRNA. These results indicate the EGF receptor is activated following the binding of PGF2 α to its receptor.²⁰⁾

Involvement of PKC δ in the transactivation of the EGF receptor was reported in ATP-stimulated

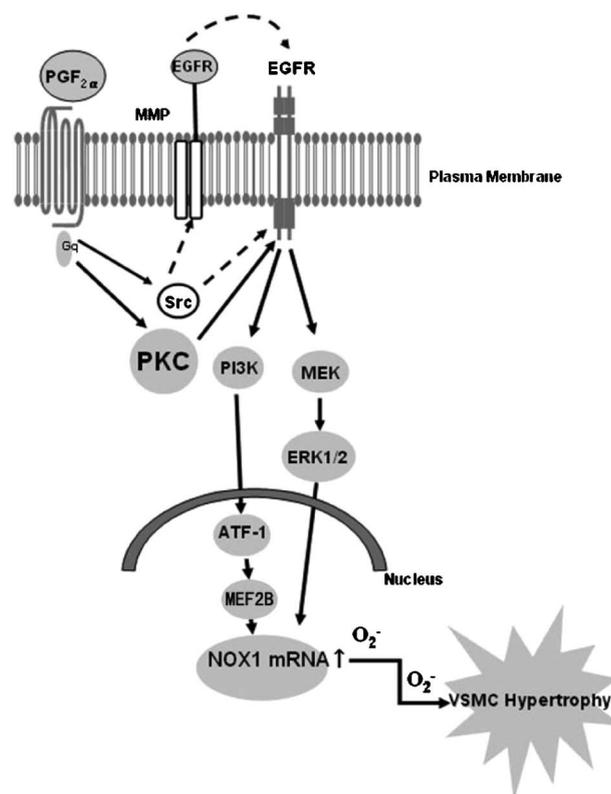


Fig. 1. The Molecular Mechanisms of PGF2 α -induced Hypertrophy of VSMC

PGF2 α exerts its biological actions through binding to its specific receptor, FP, on plasma membranes. Upon ligand binding, the FP signals to downstream molecules, PKC δ . And then the activation of Src and MMPs is involved in the transactivation of the EGF receptor by PGF2 α . Once EGF receptor is phosphorylated, there exists two independent pathways, PI3K-ATF1-MEF2B and MEK-ERK1/2. ATF-1, as a PI3K intracellular substrate, is activated. Activated ATF-1 are translocated into the nucleus to regulate NOX1 gene expression via MEF2B binding to the promoter region of rat NOX1 gene. Furthermore, the other signaling pathway including ERK1/2, which as the downstream molecule of MEK regulates positively the expression of NOX1 gene was also identified.

VSMC²¹⁾ and in angiotensin-II stimulated hepatic C9 cells.²²⁾ Similarly, involvement of PKC in up-regulation of NOX1 expression by PGF2 α was investigated by the different kinds of PKC inhibitors. For example, GF109203x, a non-selective inhibitor of PKC, dose-dependently suppressed the induction of NOX1 mRNA by PGF2 α . Whereas an inhibitor of the conventional PKC, Gö6976, and a PKC ϵ translocation-inhibitor peptide had no effect, an inhibitor of PKC δ , rottlerin, significantly attenuated the PGF2 α -induced increase in NOX1 mRNA. Gene silencing of PKC δ by RNA interference significantly suppressed the PGF2 α -induced increase in NOX1 mRNA, as well as phosphorylation of the EGF receptor, ERK1/2 and ATF-1. Moreover, the augmented synthesis of the protein induced by PGF2 α was abolished by gene silencing of PKC δ . These results suggest that PKC δ

mediated transactivation of the EGF receptor.²³⁾

Transactivation of the EGF receptor by angiotensin II is mediated by the activation of tyrosine kinases Src and Pyk2, and the ADAM family of MMPs, leading to the excision of heparin-binding EGF.²⁴⁾ Therefore, effects of inhibitors of Src and MMPs were investigated. A Src inhibitor PP2 and a MMP inhibitor GM6001 suppressed the PGF2 α -induced NOX1 expression. These results suggest that the activation of Src and MMPs is involved in the transactivation of the EGF receptor by PGF2 α , which leads to the increased expression of the NOX1 gene.²⁰⁾

Thirdly, Two Independent Pathways, PI3K-ATF1-MEF2B and MEK-ERK1/2 Are Activated following EGF Receptor Transactivation in the PGF2 α -induced Upregulation of NOX1

We demonstrated that PI3K is the upstream molecule that activates ATF-1 and induces the expression of the NOX1 gene. PI3K inhibitors, but not a MEK inhibitor, suppressed the PGF2 α -induced phosphorylation of ATF-1. On the other hand, PGF2 α -induced phosphorylation of ERK1/2 was not affected by PI3K inhibitors, or by depletion of ATF-1 with RNAi. Thus, the PI3K-ATF-1 pathway and the MEK-ERK1/2 pathway, both located downstream of the EGF receptor, are independently activated to elicit induction of NOX1.²⁰⁾

Later in 2007, we found a PGF2 α -responsive element located between -146 and -125 in the 5'flanking region contains a consensus binding site for MEF2B, to which binding of MEF2B was augmented by PGF2 α . Gene silencing of MEF2B by RNA interference significantly suppressed the expression of NOX1, while silencing of activating transcription factor ATF-1, previously implicated in up-regulation of NOX1, abolished the PGF2 α -induced expression of MEF2B. These results indicate that superoxide production in vascular smooth muscle cells is regulated by the ATF-1-MEF2B cascade through the induction of the expression of the NOX1 gene.²⁵⁾

CONCLUSION

On the whole, we make research in the field of PGF2 α intercellular signal transduction with the following assays. Based on the knowledge of signaling pathways of other vasoactive factors including angiotensin II and PDGF, we use kinds of specific inhibitors to clarify the PGF2 α pathway. In addition,

gene silencing is used to verify the above results. To elicit the molecular basis of transcriptional activation, we analyzed the promoter region of the rat NOX1 gene and subcloned different region of NOX1 promoter into a reporter vector. Electrophoretic mobility shift assay (EMSA) was carried out to identify whether the transcriptional factors binds to the sequence in the NOX1 promoter. So MEF2B is demonstrated to act as a downstream molecule of ATF-1 and increase PGF2 α -induced NOX1 transcription directly *via* binding to its promoter. We found accidentally that DPI, an inhibitor of NADPH oxidase did not only reduce NADPH activity, but also down-regulate NOX1 expression, which demonstrated the involvement of the mitochondrial respiratory chain in PGF2 α -induced upregulation of NOX1.²⁶⁾

However there are still many questions waiting for answers, such as, what is the relationship between the two independent signaling pathways following EGF receptor? Which one contributes more on the PGF2 α -induced upregulation of NOX1? Do other proteins exist as downstream molecules of ERK1/2 just like MEF2B? In order to cast more light on the PGF2 α pathways, further investigation is needed.²⁷⁾

REFERENCES

- 1) Griendling K. K., Sorescu D., Ushio-Fukai M., *Circ. Res.*, **86**, 494–501 (2000).
- 2) Irani K., *Circ. Res.*, **87**, 179–183 (2000).
- 3) Guzik T. J., West N. E., Black E., McDonald D., Ratnatunga C., Pillai R., Channon K. M., *Circ. Res.*, **86**, E85–E90 (2000).
- 4) Ushio-Fukai M., Zafari A. M., Fukui T., Ishizaka N., Griendling K. K., *J. Biol. Chem.*, **271**, 23317–23321 (1996).
- 5) Patterson C., Ruef J., Madamanchi N. R., Barry-Lane P., Hu Z., Horaist C., Ballinger C. A., Brasier A. R., Bode C., Runge M. S., *J. Biol. Chem.*, **274**, 19814–19822 (1999).
- 6) Cifuentes M. E., Rey F. E., Carretero O. A., Pagano P. J., *Am. J. Physiol. Heart Circ. Physiol.*, **279**, H2234–H2240 (2000).
- 7) Dorn G. W., II Becker M. W., Davis M. G., *J. Biol. Chem.*, **267**, 24897–24905 (1992).
- 8) Adams J. W., Migita D. S., Yu M. K., Young R., Hellickson M. S., Castro-Vargas F. E., Domingo J. D., Lee P. H., Bui J. S., Henderson S. A., *J. Biol. Chem.*, **271**, 1179–1186 (1996).

- 9) Lai J., Jin H., Yang R., Winer J., Li W., Yen R., King K. L., Zeigler F., Ko A., Cheng J., Bunting S., Paoni N. F., *Am. J. Physiol.*, **271** (6 Pt 2), H2197–H2208 (1996).
- 10) Adams J. W., Sah V. P., Henderson S. A., Brown J. H., *Circ. Res.*, **83**, 167–178 (1998).
- 11) Kunapuli P., Lawson J. A., Rokach J. A., Meinkoth J. L., FitzGerald G. A., *J. Biol. Chem.*, **273**, 22442–22452 (1998).
- 12) Rao G. N., Madamanchi N. R., Lele M., Gadiparthi L., Gingras A. C., Eling T. E., Sonenberg N., *J. Biol. Chem.*, **274**, 12925–12932 (1999).
- 13) Lambeth J. D., Cheng G., Arnold R. S., Edens W. A., *Trends Biochem. Sci.*, **25**, 459–461 (2000).
- 14) Cheng G., Cao Z., Xu X., van Meir E. G., Lambeth J. D., *Gene*, 269(1–2), 131–140 (2001).
- 15) Bánfi B., Molnár, G., Maturana A., Steger K., Hegedűs B., Demarex N., Krause K. H., *J. Biol. Chem.*, **276**, 37594–37601 (2001).
- 16) Suh Y. A., Arnold R. S., Lassegue B., Shi J., Xu X., Sorescu D., Chung A. B., Griendling K. K., Lambeth J. D., *Nature*, **401**, 79–82 (1999).
- 17) Lassegue B., Sorescu D., Szöcs K., Yin Q., Akers M., Zhang Y., Grant S. L., Lambeth J. D., Griendling, K. K., *Circ. Res.*, **88**, 888–894 (2001).
- 18) Katsuyama M., Fan C., Yabe-Nishimura C., *J. Biol. Chem.*, **277**, 13438–13442 (2002).
- 19) Kiriya M., Ushikubi F., Kobayashi T., Hirata M., Sugimoto Y., Narumiya, S., *Br. J. Pharmacol.*, **122**, 217–224 (1997).
- 20) Fan C., Katsuyama M., Nishinaka T., Yabe-Nishimura C., *FEBS Lett.*, **579**, 1301–1305 (2005).
- 21) Ginnan R., Pfeleiderer P. J., Pumiglia K., Singer H. A., *Am. J. Physiol. Cell Physiol.*, **286**, C1281–C1289 (2004).
- 22) Shah B. H., Catt K. J., *Mol. Pharmacol.*, **61**, 343–351 (2002).
- 23) Fan, C., Katsuyama, M., and Yabe-Nishimura C., *Biochem. J.*, **390**, 761–767 (2005).
- 24) Shah B. H., Catt K. J., *Trends Pharmacol. Sci.*, **24**, 239–244 (2003).
- 25) Katsuyama M., Cevik M. O., Arakawa N., Kakehi T., Nishinake T., Iwata K., Ibi K., Matsuno K., Yabe-Nishimura, C., *FEBS J.*, **274**, 5128–5136 (2007).
- 26) Katsuyama M., Fan C., Arakawa N., Nishinaka T., Miyagishi M., Taira K., Yabe-Nishimura C., *Biochem. J.*, **386**, 255–261 (2005).