Mitogen-activated protein kinases (MAP Kinases) and caveolin on obstetrics

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Tyrosine Phosphorylation of Caveolin 1 in Placental Artery Endothelial Cells During Pregnancy

During normal pregnancy, endothelial cells in the uteroplacental and fetoplacental vascular beds must undergo significant physiological adaptations to pregnancy for increasing blood flows for delivering nutrients and oxygen supplies to support the growing fetus. On the contrary, dysfunctional activation of endothelial cells is a hallmark of pregnancy complications, such as hypertension, preeclampsia, and gestational diabetes. Despite extensive studies having been performed, our knowledge regarding the causes and consequences of endothelial adaptations to normal pregnancy or dysfunctional endothelial activation during complicated pregnancies is still very limited. However, endothelial cells are subjected to the dynamic changes in enhanced pregnancy-specific redox status favoring prooxidant production due to increased metabolic activities. Although the causes of pregnancy-specific diseases such as preeclampsia are mutifactorial and their pathogenesis are unknown, one emerging hypothesis is that placental and maternal factors converge to generate oxidative stress. It causes these disease characteristics of dysfunctional activated endothelial cells and provides a reasonable rationale for the antioxidant therapy for preeclampsia.

Caveolin 1 (*CAV1*) is the product of the *caveolin 1* gene that belongs to the *caveolin* gene family composed of three different genes, i.e., *CAV1, CAV2,* and *CAV3*. *CAV1* protein is the principal residual protein component of the plasma membrane invaginations termed as caveolae. *CAV1* is highly expressed and caveolae are extremely abundant in endothelial cells.

Since the identification of *CAV1* a decade ago, extensive studies have unraveled that this cell surface organelle participates in the regulation of numerous cellular functions. It is not surprising that *CAV1*/ caveolae are required for the maintenance of normal endothelial cell functions and thus vascular tone. *CAV1* and caveolae play a pivotal role in the regulation of various aspects of endothelial cell functions, including transcytosis, potocytosis, signal transduction, proliferation and differentiation, as well as vascular permeability.

Most of endothelial cell biologies are directly or indirectly mediated by *CAV1* interactions with various membrane-associated molecules, such as receptors, neutral lipids, and protein kinases, etc. A tyrosine residue (Tyr14) located at the NH2-terminus of *CAV1* protein can be rapidly phosphorylated in response to a number of cellular stresses, including oxidative stress and polypeptide growth factors, including insulin/insulin-like growth factor-1, platelet-derived growth factor, epidermal growth factor, and vascular endothelial growth factor. Although the functional consequences of Tyr14 phosphorylation of *CAV1* are very much unknown, Tyr14

phosphorylated *CAV1* is involved in regulating endothelial cell activation and migration. One critical aspect of *CAV1* function in the vasculature is that *CAV1* functions as a negative regulator of endothelial nitric oxide synthase. In the uteroplacental and fetoplacental circulations, we believe that *CAV1* and caveolae signaling play an important role in the regulation of nitric oxide-mediated vasodilatation and angiogenesis in the uterine and placental vascular beds essential for upregulating uterine and placental blood flows to meet the progressive needs for the growing fetus during pregnancy.

Reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2) are biologically important O_2 derivatives formed from univalent oxygen reduction in the presence of free electrons during the reduction-oxidation (redox) cycle of O_2 metabolism. Under physiological conditions, ROS are produced at low concentrations controlled tightly by a balance between pro-oxidant production and antioxidant capacity. They participate in cell signaling events mediating almost all aspects of cellular activities and reactivities.

In living cells, the most common ROS is the unstable $O_2^{\cdot-}$, which is rapidly reduced to the relatively stable H_2O_2 via the dismutation reaction catalyzed by superoxide dismutase (SOD) under physiological conditions. A large body of evidence has shown that ROS are important in the maintenance of vascular biology because of their redox potential. However, under pathophysiological conditions, the balanced intracellular redox state shifts to the left, overwhelming pro-oxidant production, thus leading to the generation of excess ROS, which is referred to as oxidative stress. Of interest to vascular biology is that, when excess $O_2^{\cdot-}$ forms, a significant portion of $O_2^{\cdot-}$ formed reacts with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$), which may cause vasoconstriction and vascular damage in the placenta.

ROS are ubiquitous reactive molecules found in the environment and in all biological systems. Thus, all living aerobic organisms are subjected to continuous threats originated from not only exogenous but also endogenous ROS generated oxidative stress. A perfect example of this phenomenon is the mammalian pregnancy process, in which the mother and the developing fetus must make gestational age-dependent cellular changes adaptive to the dynamically changing redox state due to the unavoidable generation of excess ROS originated from enhanced pregnancy-specific O_2 metabolism. For example, in comparison with nonpregnant women, the maternal blood concentrations of lipid peroxides, a marker of oxidative stress derived from ROS reaction, are much greater in normal pregnant women. From this standpoint, normal pregnancy is a physiological oxidative stress condition that the mother and fetus can tolerate. However, pregnant women with hypertension, preeclampsia, and gestational diabetes have further elevated levels of circulating lipid peroxides, which indicate the mother and fetus are even more subjected to oxidative stress.

Although the origin of maternal and fetal ROS is poorly defined, available evidence suggests that maternal blood lipid peroxides and other oxidative stress markers are primarily originated from the placenta during pregnancy. To this end, fetoplacental and possibly uteroplacental endothelial cells are direct targets of placenta-derived ROS during vascular endothelial adaptations to normal pregnancy and complicated pregnancies resulting from dysfunctional endothelial activation. Furthermore, it is also noteworthy that endothelial adaptation to normal pregnancy and dysfunctional endothelial activation during complicated pregnancies are reversible postpartum.

It is impossible to conclude from current data if the dose dependency of H_2O_2-induced rapid *CAV1* Tyr14 phosphorylation is physiologically and/or pathophysiologically relevant during pregnancy. However, it has been postulated that there might be threshold concentrations for ROS to function as either participating in the regulation of intracellular normal physiology or to be as toxic byproducts of O_2 metabolism. The rapid *CAV1* Tyr14 phosphorylation upon exposure to H_2O_2 at lower concentration is transient

and is reversible rapidly upon the withdrawal of H_2O_2. At a much higher concentration, H_2O_2 was less effective than the lower ones in the stimulation of *CAV1* phosphorylation and the former induced cell death.

It is believed that these unique features of *CAV1* phosphorylation upon oxidative stress exposure implicate an important role of *CAV1* phosphorylation in the regulation of placental endothelial cell functions during normal or complicated pregnancies. In complicated pregnancies, dysfunctional activated endothelium such as preeclampsia and gestational diabetes, is somewhat recoverable upon delivery or removal of the placenta. The reversibility of *CAV1* Tyr14 phosphorylation by oxidative stress makes *CAV1* a potential marker of the dysfunctional activation of endothelial cells.

Thus, it would be interesting and important to test if placenta endothelial cells from complicated pregnancies are associated with increased *CAV1* tyrosine phosphorylation in vivo compared with normal pregnancies.

More importantly, revealing a cause-effect relationship between excess ROS and its functional consequences might provide insights for exploring the etiology and pathogenesis of preeclampsia because excess ROS generated from abnormal hypoxia/reoxygenation of the placenta thus impairing placentation is one of most likely causes of preeclampsia.

MAPKs as Promising Therapeutic Target of Vascular Remodeling especially During Pregnancy

The protective effects of estrogen are related to favorable changes in the plasma lipid profile, to the inhibition of vascular smooth muscle cell (VSMC) proliferation and migration, to the relaxation of coronary vessels through endothelial NO synthase (eNOS) activity, and to the reduction of platelet and monocyte aggregation, tumor necrosis factor-release, and extracellular matrix synthesis. Estrogen can bind 2 estrogen receptors (ERs), ERα and ERβ , which are expressed in all vascular cell types. The classic genomic mechanism, or long-term effect of estrogen on vascular tissues, is dependent on a change in gene expression. Most recently, a second mechanism related to the direct effect of estrogen has been identified. Several studies have demonstrated that 17-estradiol (17E) can activate many intracellular signaling responses.

Extracellular stimuli lead to a wide variety of cellular responses such as cellular phenotypic change, growth, apoptosis, migration, or gene expressions. The activation of intracellular signal transduction pathways is the first important molecular event underlying cellular responses. The Mitogen-activated protein kinases (MAPK) cascade plays a central role in the cellular signal transduction pathway in response to vascular stimuli.

In vertebrates, multiple isoforms of MAPKs have been identified at least four subfamilies, *i.e.* extracellular signal-regulated kinases (ERKs), $p38^{MAPK}$, and the Jun N-terminal kinases (JNK) or stress-activated protein kinases as well as BMK/ERK5. Several studies have shown that estrogen treatment may have beneficial effects on the cardiovascular system by reducing postinjury neointimal formation and by improving some aspects of endothelial function. It has previously demonstrated that a local delivery of 17E on a porcine coronary angioplasty reduces the degree of restenosis by up to 50% and improves the reendothelialization, eNOS expression, and vascular healing. MAPK are protein serine/threonine kinases and play a critical role in cell differentiation, growth and apoptosis, and the regulation of various transcription factors and gene expressions. MAPKs are involved in chemotactic and mitogenic activity in a variety of cell types. ERK, JNK, and $p38^{MAPK}$ have differential roles in the pathophysiology of vascular remodeling regarding the molecular mechanism of smooth muscle cell proliferation, endothelial function and the role in gene expression of factors. It was proposed that the estrogen has beneficial effect through influence to MAPK activities in variable cells.

The treatment with estrogen stimulates uterine artery endothelial cells (UAECs) but reverses VSMC phosphorylation of p42/44 and $p38^{MAPK}$. And these effects are at least partially ER dependent.

Substantial rises (20- to 80-fold) in uterine and placental blood flows during pregnancy are fundamental to fetal development and maintain normal maternal physiologic functions because progressively increased blood circulation is required for providing sufficient nutrients and oxygen supplies to support the growth of the fetus and for exhausting respiratory gases and metabolic wastes from fetus to mother.

Estrogen activates eNOS in UAECs

Estrogen is a potent vasoactive hormone that can initiate very rapid vasodilatation in various vascular beds and tissue perfusion during the follicular phase of the menstrual cycle and during human pregnancy in which both physiological conditions are with significantly elevated circulating estrogen levels. This rapid vasodilatory effect of estrogen in the uterus has shown to be mediated largely by artery endothelial production of the potent vasodilator nitric oxide (NO), which is primarily derived from the conversion of l-arginine to l-citrulline by the Ca^{2+}-dependnent endothelial NO synthase (eNOS). However, the molecular mechanism by which estrogen stimulates eNOS to produce NO in uterine artery endothelium is unknown.

Recent studies say that chronic estrogen treatment increases eNOS protein expression in uterine artery endothelium *in vivo*. Increased eNOS protein expression is expected to stimulate NO production. However, this cannot fully explain the rapid timing of estrogen-induced rises in uterine and systemic blood flows. Obviously, mechanism(s) other than gene expression may play an even more important role in stimulating eNOS to produce NO during estrogen-induced vasodilatation because gene expression requires hours to days to occur.

On estrogen stimulation an array of rapid cellular responses, including mobilization of intracellular Ca^{2+} and activation of MAPK and protein kinase B/Akt, can occur within seconds to minutes. It is therefore possible that estrogen may use some of these rapid signaling pathways alternatively to activate eNOS resulting in NO mediated uterine vasodilatation.

eNOS protein possesses multiple putative phosphorylation sites, which can be phosphorylated by various protein kinases including Akt and ERK2/1. On estrogen stimulation, Akt and ERK2/1 are rapidly phosphorylated that in turn activate eNOS. It seems that phosphorylation of ERK2/1 induced by physiological stimuli correlates to elevated NO production strongly and positively, which suggest that activated ERK2/1 may be stimulatory for eNOS activity.

It shows that estrogen rapidly induce ER-dependent activation of eNOS and NO production and phosphorylation of ERK2/1 with no apparent Akt phosphorylation or measurable intracellular Ca^{2+} mobilization. So, activation of ERK pathway may play an important role in acute activation of eNOS by estrogen.

Antimitogenic and Antichemotactic Effects of estrogen in VSMCs

In animal experiments, PDGF-BB has been associated with SMC proliferation and migration. PDGF- BB can induce the phosphorylation of p42/44 and $p38^{MAPK}$ within 5 and 30 minutes, respectively, in VSMCs. It is well established that treatment with estrogen inhibited the proliferation and migration of VSMCs stimulated by PDGF-BB in in vitro and in vivo experiments. Estrogens mediated their inhibitory effects on VSMCs by reducing p42/44 and $p38^{MAPK}$ activity through the nongenomic effects of estrogen.

The pretreatment with ER antagonists to VSMCs before 17E reversed the effect of 17E, ie, prevention of the phosphorylation of p42/44 and $p38^{MAPK}$ induced by PDGF-BB. It suggested the antimitogenic and antichemotactic effects of 17E on PSMCs are, at least, ER dependent.

Estrogen Promotes Proliferation and Migration of AECs

A previous study has noted that the administration of estrogen in healthy young men is associated with enhanced arterial endothelial function. And the local delivery of 17E improves reendothelialization and eNOS expression. In the recent study, we proposed that 17E increases reendothelialization by increasing the proliferation and migration of AECs. To examine the nongenomic effects of 17E on AEC proliferation and migration, it was evaluated the MAPK activity of these cells after a brief administration of 17E. The results demonstrated that treatment of AECs with 17E increased p42/44 and $p38^{MAPK}$ phosphorylation within 5 and 30 minutes of stimulation, respectively. These results support that estrogen can preserve the actin cytoarchitecture during metabolic stress, induce the migration of endothelial cells by stimulation of the p42/44 and $p38^{MAPK}$ pathway. To determine whether these rapid activations of p42/44 and $p38^{MAPK}$ by 17E are ER dependent or independent, pretreatment of AECs with ER antagonists reverses the phosphorylation of p42/44 and $p38^{MAPK}$ mediated by 17E.

In conclusion, an acute administration of 17E activates p42/44 and $p38^{MAPK}$, thus promoting the proliferation and migration of AECs, and in contrast, it inhibits these events in VSMCs. These results suggest that the beneficial effects of treatment with 17E on vascular system may be explained by a reduction of VSMC migration and proliferation combined with positive endothelial cell migrating and proliferating activity. These effects of 17E appear to be at least partially ER dependent.

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