Angiotensin II and Cell Cycle Regulation
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Abstract—Angiotensin II has emerged as an important growth factor for vascular, cardiac, and renal cells. Depending on the specific cell type and presence of other growth factors, angiotensin II induces proliferation (replication of DNA with subsequent successful division of cells), hypertrophy (increase in cell size, cell protein, and mRNA content without DNA replication), apoptosis (programmed cell death), or differentiation. Such angiotensin II-mediated modulation of growth process may underlie various pathophysiological processes such as atherosclerosis, vascular and cardiac remodeling, and progression of chronic renal disease. Clearly, angiotensin II-induced proliferation requires complete cell progression through the various steps of the cell cycle. In contrast, cells undergoing angiotensin II-mediated hypertrophy are arrested in the G1-phase. Upregulation of cell cycle-dependent kinase inhibitors (eg, p27Kip1) plays an important role in this process. Although accumulating evidence suggests that apoptosis is cell cycle-dependent, only few data are currently available concerning the interaction of angiotensin II with the cell cycle machinery in apoptosis. We review the various angiotensin II-mediated growth processes and their relationship to events governing cell cycle regulation. (Hypertension. 2004;43:1-6.)

Key Words: renin-angiotensin system ■ hypertrophy ■ apoptosis

Angiotensin II (Ang II) was originally discovered as a potent vasoconstrictor. However, research in the past several decades has provided ample evidence that Ang II modulates cell growth.1–4 Depending on the cell type, subtype of Ang II receptors, and the presence of other growth factors and cytokines, Ang II may either stimulate growth (proliferation, hypertrophy) or act as a growth suppressor (apoptosis, antiproliferation with induction of differentiation). Although Ang II is an important growth modulator of blood vessels and renal organogenesis during development, much has been learned from its role in disease. Ang II modulates cell growth in various diseases such as atherosclerosis, vasculitis, hypertension-induced injury, cardiac hypertrophy, compensatory renal hypertrophy, and proliferation of renal cells during nephritis.

Basic Mechanisms of Cell Cycle Regulation
Several comprehensive reviews provide details of basic mechanisms of cell cycle regulation.5,6 Cell growth occurs in 2 main phases: interphase and mitosis. Interphase may be further divided into G1, S, and G2 phases (Figure 1). Nondividing cells are in a dormant G0 phase and will re-enter the G1 phase after appropriate stimulation. They then pass through G2 into the S phase, where DNA replication takes place and, after progressing through G2, enter mitosis.

The transition points between the various phases of the cell cycle are regulated by kinase activity of a distinct holoenzyme that is composed of 2 subunits: cyclin and appropriate cyclin-dependent kinase (CDK). Different CDKs are active at different points of the cell cycle (Figure 2). The activities of the cyclin-CDK complexes depend on phosphorylation. The absolute concentration of CDK protein does not normally change, whereas cyclins accumulate through increased synthesis and are subsequently destroyed. The cyclin-CDK complex itself exhibits kinase activity necessary for the phosphorylation of various substrates resulting in cell cycle progression.

Entry into G1 and eventual progression into the S-phase may ultimately determine whether the cell undergoes G1 phase arrest with accompanying hypertrophy or whether the cell completes mitosis leading to proliferation (Figure 1). Specific G1 cyclins have been identified, such as cyclins D1–3, which associate with CDKs 4 and 6, and cyclin E, which associates with CDK2. Cyclins of the D type are responsible for the growth factor-induced transition from G0 into G1. One of the most important substrates of cyclin D-CDK is the 110-kDa protein product of the retinoblastoma gene (Rb). The cell cycle machinery is connected to the regulation of transcription of various target genes through the phosphorylation pattern of Rb. Cyclin E expression occurs after that of D-type cyclins in the late G1 phase and plays a pivotal role in G1/S transition. However, recent data suggest that the function of CDK2 may not be quite so straightforward. For instance, the CDK2 knockout mouse is fully viable and has no cell cycle abnormalities.5 In contrast, mice lacking cyclins E1 and E2 exhibit an inability of G1-phase cells to reenter the cell cycle. Apparently, essential functions of cyclin E are not CDK2-dependent.5
Angiotensin II-induced proliferation

The first studies demonstrating proliferative effects of Ang II were performed in bovine adrenocortical cells. In the kidney, Ang II stimulates proliferation of cultured mesangial cells. Ang II also induces proliferation of endothelial cells depending on the cell type. Second, Ang II infusion into naive animals causes systemic hypertension. Double-transgenic rats harboring human renin and angiotensinogen genes and animals with 2 kidneys with 1 clipped are also hypertensive. Hypertension can directly modulate growth through mechanical stretch receptors. To separate effects of systemic hypertension from a more direct growth-stimulatory property of Ang II on a cellular level is extremely difficult. Cyclic stretch in cultured mesangial cells activates various kinases including protein kinase C, protein tyrosine kinase, p44/42 mitogen-activated protein kinase, and stress-activated protein kinase. Activation of this signal transduction pathway stimulates proliferation. To make the relationship between Ang II and hypertensive mechanical stretch even more complex, stretch stimulates Ang II release and upregulates Ang II receptors.

Finally, Ang II induces other vasoactive factors such as endothelin, aldosterone, and increased sympathetic nervous system activation. All these factors can themselves exhibit growth-modulating effects. Such effects are best characterized for endethelins, that are promitogenic factors for many cells. Furthermore, the β-adrenergic agonist isoproterenol stimulates tubular proliferation. Consequently, many researchers have used cell culture systems to better-characterize potential effects of Ang II on the cell cycle. Ang II induces, in certain cells, proliferation that requires complete progression through the cell cycle with successful mitosis (Figure 3). However, Ang II stimulates cellular hypertrophy. Moreover, Ang II has been implicated in mediating apoptosis and cell differentiation (Figure 3).

Ang II-Induced Proliferation

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from different vessels beds and renal distal tubular cells. Although cell cycle analysis was not performed in these earlier studies, an increase in cell number after Ang II challenge presumably reflects progression through all phases of the cycle with successful completion of mitosis. Ruiz-Ortega and Egidio performed cell cycle analysis in renal interstitial fibroblasts treated with Ang II. Their data show that when the peptide was added to quiescent fibroblasts, the cells re-entered and progressed through the cell cycle.

Ang II (200 ng/min) infusions with osmotic minipumps into rats for 14 days leads to a strong proliferation of vascular smooth muscle cells (VSMCs) as determined by incorporation of [3H]thymidine and autoradiography. Mervaala et al investigated growth effects in double-transgenic rats harboring human renin and human angiotensinogen genes. These animals have high local tissue Ang II levels, and organ injury is partly independent of hypertension. There was a significant increase in Ki-67-positive cells in the heart and kidney in double-transgenic rats; however, the exact cell type remains unclear. Moreover, these rats revealed profound perivascular monocyte/macrophage infiltration, suggesting that Ang II mediates proliferation and migration of monocytes/macrophages. Interestingly, p27Kip1 has been recently implicated in reducing migration of cells. Because enhanced local Ang II concentrations in the double-transgenic rats presumably lead to downregulation of p27Kip1 in cells undergoing proliferation, this result would also increase their migratory activity.

Diep et al studied expression of cell cycle proteins in Ang II-infused rats. Ang II infusion (120 ng/kg per minute) for 7 days significantly increased [3H]thymidine incorporation into mesenteric arteries. This proliferative response was associated with a strong protein expression of cyclin D4 and CDK4. In contrast, expression of p21Cip1 and p27Kip1 was suppressed after Ang II infusion. These findings are not necessarily surprising because progression into the S phase of the cell cycle in which [3H]thymidine incorporation occurs requires passage through G1, with activation of cyclin D-CDK4 complexes and concomitant downregulation of CKIs. Interestingly, p27Kip1 expression is increased in intimal VSMCs after 3 weeks, but not immediately after balloon injury. This response is presumably an intrinsic mechanism to limit proliferation of VSMCs. Whether Ang II is directly involved in the upregulation of p27Kip1 remains unclear.

Some of the Ang II-related proliferative effects may be mediated by the transcription factor, nuclear factor (NF)-kB. NF-kB is induced by Ang II in vascular and renal cells. NF-kB is also activated in the human renin-angiotensinogen double-transgenic rats. The interactions of NF-kB with proteins of the cell cycle are multiple. For example, NF-kB activation stimulates the transcription of cyclin D1.

**Ang II and Hypertrophy**

Hypertrophy is defined as an increase in cell size, RNA content, and protein synthesis, without concomitant changes in cellular DNA content. Accumulating evidence shows that hypertrophy is an active process in which glomerular cells re-enter the cell cycle from G0. However, cells undergoing hypertrophy do not progress into S phase, but instead are arrested in G1. This explains why hypertrophy and proliferation are exclusive at a single cell level. In addition to cell cycle-dependent hypertrophy, there is also evidence that hypertrophy of certain cells can be induced by cell cycle-independent mechanisms, because of an inhibition of protein degradation.

Geisterfer et al reported in 1988 that Ang II induces hypertrophy, but not proliferation in confluent cultured rat aortic smooth muscle cells. Cell cycle analysis revealed that VSMCs treated with 10⁻⁶ M Ang II were arrested in the G1-phase and did not reach the S-phase. The hypertrophic effects of Ang II on VSMCs were confirmed in other studies. Ang II stimulates the expression of immediate early genes. Because these genes are expressed in the G1-phase, Ang II-mediated expression of immediate early genes reflects G1-phase re-entrance of resting cells, but not necessarily progression into the S phase. Thus, Ang II-mediated induction of immediate early genes is concordant with G1-phase arrest and hypertrophy. Both the mitogen platelet-derived growth factor (PDGF)-BB and Ang II stimulated the accumulation of G1-phase cyclins with similar kinetics in VSMCs. Although both factors increased the enzymatic activity of CDK4, only PDGF stimulated CDK2 activity in the late G1 phase, despite a similar protein expression of both CDKs. The lack of activation of CDK2 in Ang II-treated VSMC was associated with a failure of p27Kip1 repression, G1-phase arrest, and hypertrophy. The p27Kip1 antisense oligonucleotides attenuated the Ang II-mediated G1-phase arrest and lead to proliferation.

More than a decade ago, we discovered that Ang II induces hypertrophy of cultured renal proximal tubular cells, as determined by stimulated protein synthesis and increased cell size without accompanying DNA synthesis. The hypertrophic action of Ang II is mediated through high-affinity AT1 receptor. However, intact AT2 receptors are also important for hypertrophy of cardiac cells. Ang II-treated cells are arrested in the G1-phase and do not progress into the S-phase. Mitogens such as epidermal growth factor and Ang II induce early immediate genes and Hox genes in proximal tubular cells. This finding suggesting that the hypertrophic action of Ang II and the mitogenic action of epidermal growth factor share similar effects on the induction of pattern of G1-phase genes.

What arrests Ang II-treated cells in the G1 phase? We found that Ang II stimulates the transcription and biosynthesis of transforming growth factor-β (TGF-β) in proximal tubular cells. The Ang II-mediated hypertrophy partly depends on this induction and the autocrine action of TGF-β, because a neutralizing anti-TGF-β antibody abolished the Ang II-induced hypertrophy of proximal tubular cells. Findings by Franch et al demonstrated that application of exogenous TGF-β prevented S-phase entry of proximal tubular cells incubated with epidermal growth factor and are in excellent agreement with these observations. We discovered that Ang II strongly stimulates expression of p27Kip1 protein, but not mRNA in cultured proximal tubular cells. To better characterize a functional role of p27Kip1 in Ang II-mediated hypertrophy, we recently isolated and characterized proximal tubular cells from p27Kip1⁻/⁻ mice. In contrast
to p27Kip1+/- tubular cells, Ang II facilitated cell cycle progression of p27Kip1+/- tubular cells without inducing hypertrophy. Ang II activated cyclin D-CDK4 kinase activity in p27Kip1 +/-/+ and +/- proximal tubular cells but stimulated cyclin E-CDK2 activity only in wild-type cells, suggesting that p27Kip1 inhibits this complex. In the presence of Ang II, reconstituting p27Kip1 expression in +/- tubular cells using an inducible expression system restored G1-phase arrest and the hypertrophic phenotype. These findings are convincing evidence that p27Kip1 is required for Ang II-induced hypertrophy of proximal tubular cells.

How does Ang II increase p27Kip1 expression in tubular cells? We have studied this process in detail. Ang II increases through upregulation of p22phox, a subunit of the membrane-bound NAD(P)H-oxidase, the intracellular concentration of reactive oxygen species. This increase in oxygen radicals leads to phosphorylation and activation of the mitogen-activated protein kinases Erk 1,2 that in turn phosphorylates p27Kip1 at serine residues. The p27Kip1 phosphorylation at serine/threonine residues leads, in contrast to tyrosine phosphorylation, to an increased stability and less degradation through upregulation of p22phox, a subunit of the membrane-bound NAD(P)H-oxidase, the intracellular concentration of reactive oxygen species. This increase in oxygen radicals leads to phosphorylation and activation of the mitogen-activated protein kinases Erk 1,2 that in turn phosphorylates p27Kip1 at serine residues. The p27Kip1 phosphorylation at serine/threonine residues leads, in contrast to tyrosine phosphorylation, to an increased stability and less degradation through upregulation of p22phox, a subunit of the membrane-bound NAD(P)H-oxidase, the intracellular concentration of reactive oxygen species. This increase in oxygen radicals leads to phosphorylation and activation of the mitogen-activated protein kinases Erk 1,2 that in turn phosphorylates p27Kip1 at serine residues. The p27Kip1 phosphorylation at serine/threonine residues leads, in contrast to tyrosine phosphorylation, to an increased stability and less degradation through upregulation of p22phox, a subunit of the membrane-bound NAD(P)H-oxidase, the intracellular concentration of reactive oxygen species.

Interestingly, inhibiting cyclin A-CDK2 activation, reduced apoptosis in p27Kip1-/-/- cells. In apoptotic p27Kip1-/-/- mesangial cells, cyclin A-CDK2 was also activated without a preceding increase in cyclin E-CDK2 activity, suggesting that uncoupling of CDK2 activity from the scheduled sequence of cell cycle protein expression may lead to an inappropriate and premature initiation of G1/S phase transition, causing the cell to respond by apoptosis rather than proliferation.

Ang II induces apoptosis under certain conditions. Renal tubular apoptosis is a prominent feature of diabetic Ren-2 rats that overexpressed renin and Ang II. Transgenic rats harboring human renin and angiotensinogen reveal an increased rate of apoptosis in the heart and kidney. It has been suggested that activation of AT2 receptors is responsible for proapoptotic effect of Ang II, but this issue is controversial because AT1 receptor antagonists also attenuated apoptosis in certain models.

Overexpression of AT2-receptors in fibroblasts had antiproliferative effects without causing apoptosis. This effect was associated with an inhibition of CDK4 and cyclin E expression. CKI expression was not influenced in the AT2-receptors expressing fibroblasts. Ang II has antiproliferative effects without causing apoptosis in PC12 cells, a pheochromocytoma cell line that exclusively expresses AT2-receptors. Antiproliferative effects of Ang II in PC12 cells are not associated with apoptosis, but represent a differentiation process. The p27Kip1 expression is upregulated in this system (Wolf et al, unpublished observations, 2004). Recently, a novel zinc homeodomain enhancer protein (Zfhep) induced by Ang II via AT1 receptors has been characterized. Zfhep expression may link AT2-receptor signaling and more downstream events such as cell cycle regulation; however, the exact relationship of these processes is currently unknown. The physiology and pathophysiology of the AT1 subtype receptor including potential signal transduction pathways is subject of intensive ongoing research and could not be reviewed here.

Conclusions

In vitro and in vivo studies have provided clear evidence that Ang II exerts growth stimulatory actions. Depending on the target cell and presence of other growth factors, Ang II may induce either proliferation or hypertrophy. That various cyclin-CDK complexes must be activated in proliferation and
CKIs should be suppressed by Ang II is not surprising. Cells undergoing Ang II-induced hypertrophy re-enter the cell cycle from G₀ but do not progress into the S phase. Ang II-mediated CKI inductions such as p27(kip1) play a pivotal role in this cell cycle arrest. In some systems, Ang II mediates apoptosis. Although accumulating data suggest that apoptosis requires active cell cycle-dependent processes, few data exist concerning the association of Ang II-induced apoptosis and regulation of cell cycle events. Finally, Ang II could exhibit antiproliferative effects associated with differentiation of cells rather than representing apoptosis. That these differences in Ang II-mediated effects could be exclusively explained by activation of different Ang II receptors or different signal transduction systems is unlikely. For example, depending on the cell type, hypertrophy and apoptosis could be mediated by either AT₁- or AT₂-receptor activation. Improved signal transduction systems is unlikely. For example, depend-

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