Angiotensin II and Cell Cycle Regulation

Gunter Wolf, Ulrich O. Wenzel

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

A ngiotensin 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 G1 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 G0-phase cells to reenter the cell cycle. Apparently, essential functions of cyclin E are not CDK2-dependent.5
So-called CDK inhibitors (CKIs) attenuate the G1-phase cyclin-CDK complex kinase activity. CKIs are relatively small molecules that bind to cyclin-CDK complexes. Two main classes of CKIs exist, the INK4 family that inhibits only D-type cyclin-CDK complexes and the Cip/Kip family that binds CDK 2, 4, and 6 (Figure 2). The p21Cip1, which is the founding member of the Cip/Kip family of CKIs, is transcriptionally regulated. Data suggest that CDK inhibition by p21Cip1 is determined by the fraction of kinase complexed with the inhibitor and not by the stoichiometry of p21Cip1 bound to CDK or the phosphorylation state of the CKI. The carboxyl-terminal segments of p21Cip1 and of p27Kip1 are truncated by caspase in apoptotic cells. Thus, CKI regulation also plays a protective role in apoptosis.

The p27Kip1 is expressed widely in nonproliferating cells. In contrast to p21Cip1, p27Kip1 expression is often, but not exclusively, posttranscriptionally regulated by changes in protein translation and degradation through the ubiquitin proteolytic pathway. The INK4 family (p16, p15, p18, p19, and p20) of CKIs is specific for CDK4 and CDK6.

What Growth Effects Are Mediated by Ang II?

To test a potential growth effect of Ang II in intact animals is difficult for several reasons. First, Ang II may exhibit different effects on cell growth (hypertrophy or proliferation), 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 endothelins, 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

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.
from different vessels beds and renal distal tubular cells.\textsuperscript{13,14} 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 Egido performed cell cycle analysis in renal interstitial fibroblasts treated with Ang II.\textsuperscript{15} Their data show that when the peptide was added to quiescent fibroblasts, the cells re-entered and progressed through the cell cycle.\textsuperscript{15}

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 \textsuperscript{[3}H\textsuperscript{]} thymidine and autoradiography.\textsuperscript{16} Mervaala et al investigated growth effects in double-transgenic rats harboring human renin and human angiotensinogen genes.\textsuperscript{17} These animals have high local tissue Ang II levels, and organ injury is partly independent of hypertension.\textsuperscript{17} 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.\textsuperscript{17} Moreover, these rats revealed profound perivascular monocyte/macrophage infiltration, suggesting that Ang II mediates proliferation and migration of monocytes/macrophages.\textsuperscript{17} Interestingly, p27\textsuperscript{Kip1} has recently been implicated in reducing migration of cells.\textsuperscript{18} Because enhanced local Ang II concentrations in the double-transgenic rats presumably lead to downregulation of p27\textsuperscript{Kip1} 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 \textsuperscript{[3}H\textsuperscript{]} thymidine incorporation into mesenteric arteries.\textsuperscript{19} This proliferative response was associated with a strong protein expression of cyclin D4 and CDK4.\textsuperscript{19} In contrast, expression of p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1} was suppressed after Ang II infusion. These findings are not necessarily surprising because progression into the S phase of the cell cycle in which \textsuperscript{[3}H\textsuperscript{]} thymidine incorporation occurs requires passage through G\textsubscript{1} with activation of cyclin D-CDK4 complexes and concomitant downregulation of CKIs. Interestingly, p27\textsuperscript{Kip1} expression is increased in intimal VSMCs after 3 weeks, but not immediately after balloon injury.\textsuperscript{20} This response is presumably an intrinsic mechanism to limit proliferation of VSMCs. Whether Ang II is directly involved in the upregulation of p27\textsuperscript{Kip1} remains unclear.

Some of the Ang II-related proliferative effects may be mediated by the transcription factor, nuclear factor (NF)-\kappaB. NF-\kappaB is induced by Ang II in vascular and renal cells.\textsuperscript{21,22} NF-\kappaB is also activated in the human renin-angiotensinogen double-transgenic rats.\textsuperscript{22} The interactions of NF-\kappaB with proteins of the cell cycle are multiple. For example, NF-\kappaB 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 G\textsubscript{0}. However, cells undergoing hypertrophy do not progress into S phase, but instead are arrested in G\textsubscript{1}. 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 confluently cultured rat aortic smooth muscle cells.\textsuperscript{3} Cell cycle analysis revealed that VSMCs treated with \textsuperscript{10}–\textsuperscript{7} M Ang II were arrested in the G\textsubscript{1}-phase and did not reach the S-phase.\textsuperscript{2} The hypertrophic effects of Ang II on VSMCs were confirmed in other studies.\textsuperscript{4} Ang II stimulates the expression of immediate early genes.\textsuperscript{3} Because these genes are expressed in the G\textsubscript{1}-phase, Ang II-mediated expression of immediate early genes reflects G\textsubscript{1}-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 G\textsubscript{1}-phase arrest and hypertrophy. Both the mitogen platelet-derived growth factor (PDGF)-BB and Ang II stimulated the accumulation of G\textsubscript{1}-phase cyclins with similar kinetics in VSMCs.\textsuperscript{23} Although both factors increased the enzymatic activity of CDK4, only PDGF stimulated CDK2 activity in the late G\textsubscript{1} phase, despite a similar protein expression of both CDKs.\textsuperscript{23} The lack of activation of CDK2 in Ang II-treated VSMC was associated with a failure of p27\textsuperscript{Kip1} repression, G\textsubscript{1}-phase arrest, and hypertrophy.\textsuperscript{24} The p27\textsuperscript{Kip1} antisense oligonucleotides attenuated the Ang II-mediated G\textsubscript{1}-phase arrest and lead to proliferation.\textsuperscript{24}

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.\textsuperscript{25} The hypertrophic action of Ang II is mediated through high-affinity AT\textsubscript{1} receptor.\textsuperscript{25} However, intact AT\textsubscript{1} receptors are also important for hypertrophy of cardiac cells.\textsuperscript{25} Ang II-treated cells are arrested in the G\textsubscript{1} phase and do not progress into the S-phase.\textsuperscript{25,27} 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 G\textsubscript{1}-phase genes.\textsuperscript{28}

What arrests Ang II-treated cells in the G\textsubscript{1} phase? We found that Ang II stimulates the transcription and biosynthesis of transforming growth factor-\beta (TGF-\beta) in proximal tubular cells.\textsuperscript{29} The Ang II-mediated hypertrophy partly depends on this induction and the autocrine action of TGF-\beta, because a neutralizing anti-TGF-\beta antibody abolished the Ang II-induced hypertrophy of proximal tubular cells.\textsuperscript{29} Findings by Franch et al demonstrated that application of exogenous TGF-\beta prevented S-phase entry of proximal tubular cells incubated with epidermal growth factor and are in excellent agreement with these observations.\textsuperscript{30} We discovered that Ang II strongly stimulates expression of p27\textsuperscript{Kip1} protein, but not mRNA in cultured proximal tubular cells.\textsuperscript{31} To better characterize a functional role of p27\textsuperscript{Kip1} in Ang II-mediated hypertrophy, we recently isolated and characterized proximal tubular cells from p27\textsuperscript{Kip1}−/− mice.\textsuperscript{32} In contrast...
to p27Kip1+/- tubular cells, Ang II facilitated cell cycle progression of p27Kip1+/- tubular cells without inducing hypertropy.32 Ang II activated cyclin D-CDK4 kinases 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.32 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.33 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.34,35 The p27Kip1 phosphorylation at serine/threonine residues leads, in contrast to tyrosine phosphorylation, to an increased stability and less degradation through the ubiquitin pathway.35 Atrial natriuretic peptide (ANP) that attenuates Ang II-induced p27Kip1 expression and hypertrophy of proximal tubular cells activates MKP-1, a phosphatase involved in dephosphorylation of Erk 1,2.36

Does Ang II in vivo also increase p27Kip1 expression? Infusion of Ang II into normal rats for 7 days increased formation of reactive oxygen species in tubular cells and increased p27Kip1 expression.37 Another situation in which CDK inhibitors may play a role is the hypertrophy of the nonclipped kidney in the 2-kidney, 1-clip model of hypertension.38 Glomerular p27Kip1 expression is strongly upregulated in the nonclipped kidney undergoing compensatory hypertrophy compared with clipped kidneys or controls.38

Hypertrophy of different renal cells occurs early in diabetic nephropathy. Similar to observations obtained with Ang II, growing mesangial cells in high glucose leads to p27Kip1, dependent cell cycle arrest and hypertrophy.39 This response may be, in fact, mediated by Ang II because high glucose stimulates generation of Ang II in mesangial cells.40 We tested the potential effects of short-term treatment with an ACE-inhibitor on renal CKI expression in BBdp rats, an autoimmune model of type I diabetes.41 Protein expression of p16INK4, p21Cip1, and p27Kip1 were stimulated in BBdp rats compared with nondiabetic BBdr animals.41 Enalapril treatment for 3 weeks, started after the onset of diabetes, reduced the glomerular expression of p16INK4 and p27Kip1, but not of p21Cip1.41

Ang II may also increase hypertrophy in diabetes indirectly through induction of connective tissue growth factor (CTGF). CTGF is a downstream mediator of TGF-β1. In double-transgenic rats expressing renin and angiotensinogen, a strong upregulation of CTGF expression was found in myocardial, vascular, and renal tissue.42 Because CTGF induces G1 phase arrest with hypertrophy in cultured mesangial cells through upregulation of p21Cip1 and p27Kip1,43 part of the hypertrophic effects of Ang II may be, in fact, mediated through CTGF.

Could cellular hypertrophy be dissociated from cell cycle arrest? TGF-β inhibits proliferation in the absence of p21Cip1 and p27Kip1.44 In contrast, the hypertrophic growth effects of TGF-β were significantly reduced in the absence of p21Cip1 and p27Kip1.44 This observation suggests that CKIs are a necessary prerequisite in mediating the hypertrophic response but not necessarily always by G1-phase arrest.44 How p21Cip1 and p27Kip1 exert hypertrophy independently of cell cycle regulation is currently unknown.

**Ang II, Apoptosis, and Antiproliferation**

Accumulating evidence indicates that cells undergoing apoptosis must also enter the cell cycle before exiting by apoptosis. Shankland’s group showed a marked increase in glomerular cell apoptosis in nephritic p27Kip1-/- mice compared with nephritic p27Kip1+/- controls.45 Apoptosis was also increased in tubulointerstitial cells after obstruction in p27Kip1-/- mice compared with control p27Kip1+/- mice.45 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.46 Transgenic rats harboring human renin and angiotensinogen reveal an increased rate of apoptosis in the heart and kidney.22 It has been suggested that activation of AT2 receptors is responsible for proapoptotic effect of Ang II,46 but this issue is controversial because AT1 receptor antagonists also attenuated apoptosis in certain models.48

Overexpression of AT2-receptors in fibroblasts had antiproliferative effects without causing apoptosis.49 This effect was associated with an inhibition of CDK4 and cyclin E expression.49 CKI expression was not influenced in the AT1-receptors expressing fibroblasts.49 Ang II has antiproliferative effects without causing apoptosis in PC12 cells, a pheochromocytoma cell line that exclusively expresses AT2 receptors.50 Antiproliferative effects of Ang II in PC12 cells are not associated with apoptosis, but represent a differentiation process.50 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.51 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.51 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 p27Kip1 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 understanding of Ang II-mediated cell cycle regulation is necessary to develop improved therapeutic strategies.

Acknowledgments

Original work in the authors’ laboratory was supported by the Deutsche Forschungsgemeinschaft (We 1688/4-3, Wo 460/2-7, Wo 460/12-1).

References


Angiotensin II and Cell Cycle Regulation
Gunter Wolf and Ulrich O. Wenzel

Hypertension. published online February 16, 2004;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2004/02/16/01.HYP.0000120963.09029.ca.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/