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Aldosterone as a renal growth factor

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Abstract: Aldosterone regulates blood pressure through its effects on the cardiovascular system and kidney. Aldosterone can also contribute to the development of hypertension that leading to chronic pathologies such as nephropathy and renal fibrosis. Aldosterone directly modulates renal cell proliferation and differentiation as part of normal kidney development. The stimulation of rapidly activated protein kinase cascades is one facet of how aldosterone regulates renal cell growth. These cascades may also contribute to myofibroblastic transformation and cell proliferation observed in pathological conditions of the kidney. Polycystic kidney disease is a genetic disorder that is accelerated by hypertension. EGFR-dependent proliferation of the renal epithelium is a factor in cyst development and trans-activation of EGFR is a key feature in initiating aldosterone-induced signalling cascades. Delineating the components of aldosterone-induced signalling cascades may identify novel therapeutic targets for proliferative diseases of the kidney.

1 Aldosterone as a Renal Growth Factor
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Abstract

Aldosterone regulates blood pressure through its effects on the cardiovascular system and kidney. Aldosterone can also contribute to the development of hypertension that leading to chronic pathologies such as nephropathy and renal fibrosis. Aldosterone directly modulates renal cell proliferation and differentiation as part of normal kidney development. The stimulation of rapidly activated protein kinase cascades is one facet of how aldosterone regulates renal cell growth. These cascades may also contribute to myofibroblastic transformation and cell proliferation observed in pathological conditions of the kidney. Polycystic kidney disease is a genetic disorder that is accelerated by hypertension. EGFR-dependent proliferation of the renal epithelium is a factor in cyst development and trans-activation of EGFR is a key feature in initiating aldosterone-induced signalling cascades. Delineating the components of aldosterone-induced signalling cascades may identify novel therapeutic targets for proliferative diseases of the kidney.

Introduction

The rennin-angiotensin-aldosterone system (RAAS) is a key regulator of systemic blood pressure through its direct action on the renal and cardiovascular systems [1, 2]. The release of aldosterone by the adrenal glands contributes to the homeostatic regulation of blood pressure under normal physiological conditions via a number of interlinked mechanisms including the modulation of vascular tone and the rate of electrolyte transport across target epithelia such as the distal nephron. Defective RAAS activity is a contributory factor in congenital and acquired hypertension [3]; consequently the individual receptors, enzymes and effectors of the RAAS cascade are important therapeutic targets in the treatment of this condition. The kidney is a key target organ for aldosterone action and the regulation of the rate of Na⁺ absorption by the distal nephron is an important factor influencing systemic blood pressure. The biological effects of aldosterone are initiated through the binding of the hormone to the mineralocorticoid receptor (MR), which is expressed by all aldosterone responsive tissues including the distal nephron [4] and the rennin secreting mesangial cells of the juxtaglomerular apparatus [5]. In the distal nephron, the aldosterone-induced modulation of ion transporter activity is mediated through the transcriptional effects of MR on transporter subunit and regulatory protein expression or through the phosphorylation of transporters and regulatory molecules by rapidly activated protein kinases. The combined transcriptional and signalling effects of aldosterone on the renal epithelium leads to the modulation of ion fluxes that impact upon whole body electrolyte homeostasis. The regulation of Na⁺ and K⁺ transport are held to be the most important functions for aldosterone in the kidney. However, recent evidence points to aldosterone having a more complex role in supporting renal cell differentiation and also in contributing to pathological processes in the kidney. The direct effects of aldosterone on renal pathology through its effects on MR-expressing cells in the renal tubule are adjunct to the histological damage inflicted on renal tissue by systemic hypertension.

Aldosterone modulates the growth of renal cells both in culture and *in vivo*. Evidence points to a contribution from aldosterone to normal renal tubule cell proliferation and differentiation from isolated renal stem cells [6] and also from studies *in vivo* using juvenile animal models [7]. Aldosterone may also contribute to the development of pathological conditions in the kidney through dysregulation of renal cell growth in the adult resulting in fibrosis or epithelial hyperplasia. The growth stimulatory effects of aldosterone, either hypertrophy or hyperplasia depending on the cell type investigated; result from the synergism between the transcriptional and rapid signalling responses stimulated by the hormone. The cross-talk between these two facets of aldosterone action is a prominent aspect of current research and blurs the distinction between genomic and non-genomic effects of steroids. Essentially aldosterone not only promotes the activation of protein kinase signalling cascades which contribute to cell growth but also up-regulates the expression of key signalling intermediates to amplify the sensitivity and magnitude of responses to aldosterone itself and other growth factors. The mechanisms by which aldosterone modulates the growth of renal cells will be the subject of this review, in particular the roles of the rapidly activated signalling cascades in potentiating and sustaining the transcriptional response to aldosterone in proliferating renal cells.

Aldosterone and Renal Stem Cell Differentiation

The development of each renal tubule from progenitor cells is a complex process reliant upon the co-operative actions of nephrogenic mesenchymal stem cells which give rise to the proximal tubule, loop of Henle and distal tubule, and other stem cells located in the collecting duct ampullae that form the collecting duct tubule itself. Cell proliferation following stem cell stimulation leading to the formation of S-shaped tubule bodies marks the earliest stage of nephron development in the mesenchymal parenchyma. The signalling processes which co-ordinate the two groups of stem cells to form a single nephron are poorly understood. Isolated renal stem cells from neonatal animals can be cultured using an *in vitro* serum-free perfusion system with the embryonic cells seeded between two layers of porous polyester matrix [8]. However, various combinations of typically used cell culture supplements such as epidermal growth factor (EGF), insulin, transferrin, selenium, retinoic acid, bovine pituitary extract or cholecalciferol do not stimulate tubule formation in this model [6]. Under these culture conditions the progenitor cells will develop hollow, tubular structures only in the presence of 100 nM aldosterone. These tubular structures display at least partial differentiation with the expression of *N*-acetylgalactosamine on surface glycoproteins as a marker for the collecting duct and also some degree of apical to basolateral epithelial polarity indicated by tight junction formation. The aldosterone biochemical precursors: cholesterol and pregnenolone did not mimic this effect on renal stem cells while progesterone and 11-deoxycorticosterone treatment resulted in only partial tubule development. The MR antagonist spironolactone inhibited the aldosterone-induced tubule formation while the GR agonist dexamethasone stimulated disorganized cell proliferation but no tubule formation [9]. Aldosterone thus acts as a potent differentiation factor for renal stem cells in neonatal animals. However, similar tubulogenic effects have not been observed in cells isolated from adult animals and the molecular basis of aldosterone-induced tubule differentiation has not been established to date.

Aldosterone-induced Signalling and Renal Cell Growth

The field of aldosterone-induced rapid signalling has developed greatly in recent years with the identification of the physiological roles of these signalling cascades in the cell and in the whole organism. The regulation of ion fluxes has always been seen as the key function of aldosterone in the kidney [10] and consequently attention has been focused on coupling aldosterone-induced signalling cascades to Na⁺ and K⁺ transport. It has also been known for some time that aldosterone can modulate the proliferation of MR expressing cells *in vitro* [11]. Modulation of cell growth by aldosterone; whether it is a metabolic stimulation (hypertrophy) or the stimulation of cell proliferation (hyperplasia) requires co-ordination of rapid signalling and transcriptional changes. The activation of several signalling cascades by aldosterone has been identified in cells derived from the distal nephron including the PKC α [12], PKD1 [13] and ERK1/2 mitogen activated protein (MAP) kinase [14] cascades. A number of the signalling cascades rapidly activated by aldosterone such as the Ca²⁺-dependent PKC isoforms and ERK1/2 also promote cell proliferation in response to mitogenic agonists. This begs the question of whether aldosterone modulates renal cell growth through the activation of these cascades.

Aldosterone treatment of isolated renal cortical collecting duct cells stimulates the biphasic activation of the ERK1/2 cascade with an early phase of transient activation

1 followed by a more sustained period of activation. Recent work has investigated the role
2 of aldosterone on ERK1/2 signalling *in vivo*. Dosing new born rats with the MR
3 antagonist spironolactone for 7 days resulted in an increase in apoptosis detected in
4 kidney sections [15]. This observation suggests that at least in juvenile animals,
5 aldosterone plays a role in inhibiting apoptosis. Spironolactone-induced apoptosis was
6 most prominent in the tubule cells of the renal cortex where MR is highly expressed.
7 MR antagonism resulted in a significant reduction in ERK1/2 and p38 MAP kinase
8 protein expression but a slight increase in the abundance of the mRNA of these two
9 kinases. Conversely JNK-2 expression was induced by spironolactone in the cells of the
10 glomeruli and in the cortical tubules [15]. A role for aldosterone in regulating apoptosis
11 in the adult kidney has also been described. Aldosterone stimulated both apoptotic and
12 mitogenic effects in human mesangial cells, which correlated with de-phosphorylation
13 of the Bcl-2 family protein Bad and the release of cytochrome c into the cytoplasm. *In*
14 *vivo*, aldosterone had a similar pro-apoptotic effect on rat mesangial cells which was
15 eplerenone-sensitive and correlated with an increase in systolic blood pressure and
16 albuminuria [16]. Aldosterone stimulated ERK1/2 activation within 10 min of treatment
17 in mesangial cells which was also eplerenone-sensitive and led to ERK1/2-dependent
18 cell proliferation [5]. Aldosterone is therefore involved in regulating the delicate
19 balance between proliferation and apoptosis in mature and immature renal tissues.
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25 The sustained activation of the ERK1/2 signalling cascade promotes growth factor-
26 stimulated cell cycle progression in fibroblasts. Transient activation of ERK1/2 is
27 insufficient to commit stimulated cells to complete cell division [17]. The protein kinase
28 D (PKD) family of serine threonine protein kinases are involved in modulating critical
29 cellular processes including sub-cellular trafficking, hypertrophy, apoptosis and
30 proliferation and are activated in response to diverse agonists [18]. The expression and
31 activation of PKD family proteins is pre-requisite for sustained activation of ERK1/2 in
32 response to non-steroid growth factors that act through G-protein coupled receptors [17,
33 19]. PKD family isoforms are in some way responsible for stabilizing the initial growth
34 factor-stimulated ERK1/2 activation [20]. The MR-dependent autophosphorylation of
35 protein kinase D occurs within 5 min of treating CCD cells with aldosterone [13]. The
36 rapid PKD activation coincides temporally with initial ERK1/2 activation in these cells.
37 The activation of both PKD and ERK1/2 by aldosterone are dependent on the trans-
38 activation and phosphorylation of the EGF receptor (EGFR) by c-Src tyrosine kinase
39 [13, 21, 22]. In wild-type CCD cells aldosterone stimulates ERK1/2 activation which
40 remains detectable 2 hours after treatment. In CCD cells suppressed in PKD1
41 expression, aldosterone stimulates only a transient activation of ERK1/2 (McEneaney et
42 al. in press). Aldosterone promotes the growth of sub-confluent CCD cells but not of the
43 PKD1 suppressed cells (Fig. 1), consequently the behaviour of aldosterone as a renal
44 growth factor is at least partially dependent on PKD1 activation.
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51 **Aldosterone-induced Signalling and Nephropathy**

52 Abnormal cell growth is a factor in the progression of pathogenic renal conditions such
53 as chronic kidney disease, polycystic kidney disease and diabetes associated
54 nephropathy. The growth promoting effects of aldosterone on renal tubule cells has
55 been proposed as a factor contributing to the rate of progression of these conditions.
56 However, the pathological effects of circulating aldosterone can only be one component
57 of a multi-factorial process which displays chronic progression leading to renal damage.
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1 Progressive tissue deterioration results from cell growth dysregulation and extracellular
2 matrix deposition leading to fibrosis and inflammation. Animal experiments and human
3 clinical trials have demonstrated an additive effect of aldosterone antagonism with
4 RAAS blockade in attenuating renal damage. MR antagonism in combination with
5 angiotensin-converting enzyme (ACE) inhibitors attenuates the progression of diabetic
6 and non-diabetic nephropathies [23]. A diabetic rat model revealed that the MR
7 antagonist eplerenone in combination with the ACE inhibitor, enalapril enhanced
8 glomerular filtration and suppressed glomerulosclerosis with a concurrent reduction in
9 TGF- β 1, Collagen type-IV and plasminogen activator factor-1 expression [24].
10 Aldosterone also stimulates NADPH oxidase-dependent production of reactive oxygen
11 species in renal cells that accelerates fibrosis that can be attenuated using eplerenone
12 [25]. The NF κ B transcriptional pathway is also activated in response to aldosterone in
13 renal principal collecting duct cells however this was mediated by SGK activity rather
14 than the stimulation of MAP kinases. The transcription of pro-inflammatory cytokine
15 genes such as IL-1 β and IL-6 are up-regulated by NF κ B [26]. The activation of NF κ B-
16 dependent transcription in this experimental system was MR-dependent and was
17 antagonized by concurrent activation of the glucocorticoid receptor. Aldosterone
18 treatment of mesangial cells results in a rapid phosphorylation of myosin phosphatase
19 target subunit-1, a substrate for Rho kinase within 20 min of hormone treatment and
20 also results in an increase in actin polymerization [27]. The state of hypertrophy
21 stimulated by aldosterone in these cells correlates with the subsequent up-regulation
22 in the expression of collagen types I, III and IV and of α -smooth muscle actin, a marker
23 for myofibroblastic transformation. These changes, stimulated by aldosterone could be
24 blocked by eplerenone and the Rho kinase inhibitor Y27632 demonstrating that this
25 cascade plays a critical role in the differentiation effects of aldosterone.
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33 **Aldosterone, EGFR and Polycystic kidney disease**

34 The aberrant stimulation of cell proliferation in the epithelium of the distal nephron is
35 one factor in the development of polycystic kidney disease [28]. Susceptible individuals
36 have a genetic predisposition to developing autosomal recessive polycystic kidney
37 disease (ARPKD) and autosomal dominant polycystic kidney disease (ADPKD)
38 through mutations in the genes encoding the fibrocystin or polycystin 1 and 2 genes
39 respectively [29, 30]. The products of these genes are multi-functional structural
40 membrane proteins that have roles in cell polarization, tight junction integrity, ion
41 transport and signal transduction. Chronic hypertension is an exacerbating factor in the
42 progression of polycystic kidney disease [31] and controlling hypertension through the
43 administration of MR antagonists is one approach to therapeutic intervention [32].
44 Renal cyst formation and enlargement is a consequence of epithelial proliferation [33]
45 and ion transport dysregulation. The formation of cysts in ARPKD is mainly localized
46 to the MR-expressing epithelium of the distal nephron [33] and this points to a role for
47 aldosterone in promoting aberrant proliferation in the distal nephron. Silencing of the
48 ARPKD-associated fibrocystin gene in HEK293 cells produced a hyper-proliferative
49 response to EGF and resulted in over-activation of the ERK1/2 cascade following EGF
50 treatment [34]. Over-expression of Heparin-binding EGF is detected in ARPKD and
51 EGF antagonistic antibodies suppress the mitogenic activity of cleared cystic fluid [35].
52 The abundance of EGFR in cells from the renal tubule is responsive to aldosterone-
53 dependent transcription [36] and expression of EGFR is necessary to permit the
54 stimulation of rapid signalling responses by aldosterone in CHO cells [37]. The
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1 activation of ERK1/2 signalling by aldosterone in cells derived from the distal nephron
2 has been described by several groups including our own [38, 39] and this response has
3 been linked to *trans*-activation of EGFR [40]. The nuclear localization of ERK1/2
4 occurs subsequent to its activation and is consistent with its role as a regulator of
5 transcription factors. The nuclear translocation of ERK1/2 in response to the
6 aldosterone treatment of CCD cells was blocked by EGFR inhibition (Fig. 2).
7 Antagonism of the RAAS to control hypertension as a means of slowing cyst formation
8 in ADPKD is the subject of an on-going clinical investigation [41].
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11 **Conclusion**

12 Aldosterone is emerging as an important factor in regulating the growth and
13 differentiation of renal cells both *in vivo* and *in vitro*. Aldosterone has an indirect effect
14 on the pathology of renal diseases such as renal fibrosis and polycystic kidney disease
15 through the detrimental effects of systemic hypertension. Aldosterone also has direct
16 effects on renal pathology through the rapid activation of protein kinase signalling
17 cascades in cells of the renal epithelium to stimulate cell proliferation and hypertrophy
18 (Fig. 3). Pharmacological intervention in the activation of these aldosterone-induced
19 signalling cascades, particularly those coupled to EGFR *trans*-activation may provide
20 novel avenues for the treatment of chronic kidney diseases in combination with existing
21 therapies.
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Figure Legends

Figure 1

Suppression of PKD1 expression inhibits aldosterone induced CCD cell proliferation. M1-CCD cells and cells stably suppressed in PKD1 expression using a plasmid expressing a PKD1 specific siRNA were seeded at a density of 1×10^4 cells per microtitre plate well, in DMEM/F12 containing $5\mu\text{M}$ dexamethasone and 10% FBS. The cells were left to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2 . The medium was then exchanged for serum-free DMEM/F12, containing $1\mu\text{M}$ dexamethasone for 24h. Cells were treated with 10nM aldosterone or vehicle control for 72h. Following treatment, the medium was carefully removed. The cells were detached using trypsin and re-suspended in a final volume of 100 μl fresh medium. Cells were counted using a Neubauer chamber, 4 wells per treatment for each cell line. Aldosterone stimulated a two-fold increase in cell number above basal proliferation rates that was blocked by suppression of PKD1 expression.

Figure 2

ERK1/2 nuclear localization following aldosterone treatment is EGFR-dependent. Murine M1-CCD cells were maintained in serum free medium for 24 h then treated with aldosterone (10 nM) for 2 min. The sub-cellular distribution of ERK1/2 MAP kinase was determined by immuno-fluorescence using an ERK1/2-specific antibody and detected using an anti-rabbit secondary antibody conjugated to Alexa488. ERK1/2 became localized to the nucleus following aldosterone treatment. The nuclear localization of ERK1/2 in response to aldosterone was blocked by pre-incubation with the EGFR-specific inhibitor tyrphostin AG1478 ($1\mu\text{M}$). The nuclear localization of ERK1/2 following aldosterone treatment is dependent on trans-activation of EGFR.

Figure 3

Aldosterone and growth dysregulation in renal cortical collecting duct cells. Chronic renal injury occurs as a result of multiple synergistic processes. Aldosterone action can be one of these processes. The interaction between aldosterone and the mineralocorticoid receptor (MR) promotes the nuclear localization of MR where it interacts with other transcription factors (TFs) to modulate gene expression. Signalling events coupled to MR include the Src-dependent trans-activation of EGFR leading to activation of the ERK1/2 and PKD1 signalling cascades. PKD1 activation stabilizes the activation of ERK1/2, while ERK1/2 phosphorylates MR and other transcription factors to modulate their activity and contribute to cell growth responses including proliferation. Serum and glucocorticoid regulated kinase-1 (SGK) is one of the earliest detected proteins to be up-regulated by aldosterone. SGK activation promotes NF κ B-dependent transcription leading to the release of pro-inflammatory cytokines IL-1 β and IL-6 to promote renal fibrosis.

Figure 1
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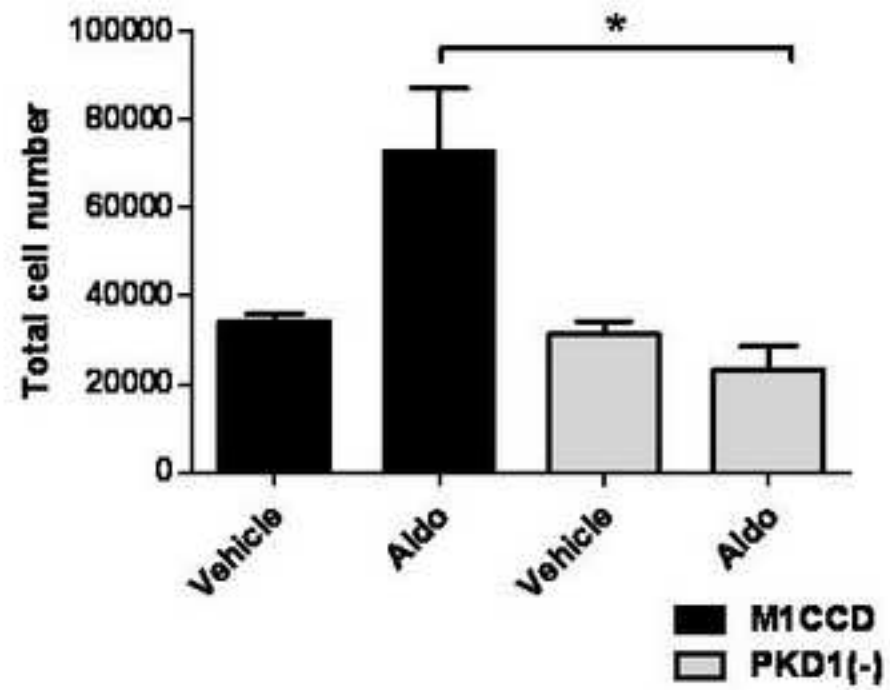


Figure 2
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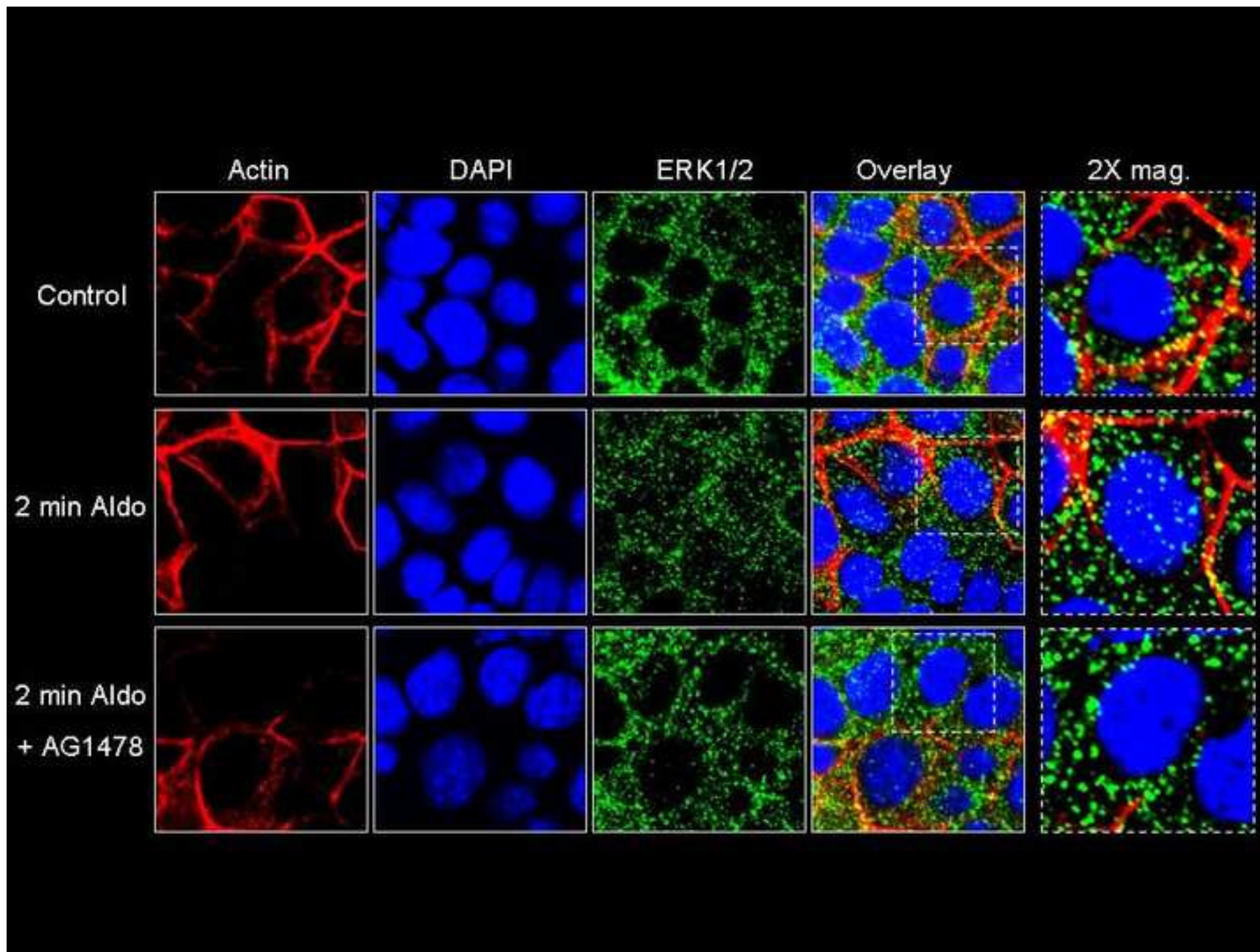


Figure 3
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Aldosterone and Renal Collecting Duct Cell Growth

