CMLS, Cell. Mol. Life Sci. 61 (2004) 2695–2703
1420-682X/04/212695-09
DOI 10.1007/s00018-004-4244-3

CMLS Cellular and Molecular Life Sciences

Hijacking epidermal growth factor receptors by angiotensin II: new possibilities for understanding and treating cardiac hypertrophy

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Abstract. Activation of the type 1 angiotensin II receptor (AT_1R) is associated with the aetiology of left ventricular hypertrophy, although the exact intracellular signalling mechanism(s) remain unclear. Transactivation of the epidermal growth factor receptor (EGFR) has emerged as a central mechanism by which the G protein-coupled AT_1R, which lacks intrinsic tyrosine kinase activity, can stimulate the mitogen-activated protein kinase signalling pathways thought to mediate cardiac hypertrophy. Current studies support a model whereby AT_1R-dependent transactivation of EGFRs on cardiomyocytes involves stimulation of membrane-bound metalloproteases, which in turn cleave EGFR ligands such as heparin-binding EGF from a plasma membrane-associated precursor. Numerous aspects of the 'triple membrane-passing signalling' paradigm of AT_1R-induced EGFR transactivation remain to be characterised, including the identity of the specific metalloproteases involved, the intracellular mechanism for their activation and the exact EGFR subtypes required. Here we examine how 'hijacking' of the EGFR might explain the ability of the AT_1R to elicit the temporally and qualitatively diverse responses characteristic of the hypertrophic phenotype, and discuss the ramifications of delineating these pathways for the development of new therapeutic strategies to combat cardiac hypertrophy.

Key words. Type 1 angiotensin II receptor (AT_1R); transactivation; EGFR; metalloprotease; HB-EGF; cardiac hypertrophy.

Introduction

Cardiac hypertrophy enables terminally differentiated cardiomyocytes to adapt to the increased workload initiated by a variety of stimuli, such as growth factors, cytokines, haemodynamic stress and G protein-coupled receptor (GPCR) agonists. Although initially a positive homeostatic mechanism, prolonged cardiac hypertrophy can be maladaptive and lead to a variety of cardiomyopathies, including left ventricular hypertrophy (LVH). LVH is a significant risk factor for cardiac morbidity and mortality [1, 2], and elucidation of the signalling pathways leading to aberrant heart growth remains a primary obstacle to rational therapeutic targets for cardiac diseases.

Angiotensin II and cardiac hypertrophy

The peptide hormone angiotensin II (AngII) and its cognate GPCR (AT_1R), are well recognised for their critical role in arterial blood pressure regulation, water balance and electrolyte homeostasis. Increasingly apparent, however, is the contribution of AngII to the development and

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maintenance of LVH. In humans and animals, angiotensin-converting enzyme (ACE) inhibitors (which prevent the synthesis of AII) and AT_1R antagonists have been shown to prevent or regress the development of hypertrophy [3–6]. AT_1Rs are upregulated in experimentally induced cardiac hypertrophy [7–9], and AngII has been shown to cause hypertrophy by direct action on cardiomyocytes; stimulation of isolated cardiomyocytes leads to the induction of a characteristic hypertrophic phenotype, including protein synthesis, cell growth and re-expression of a foetal gene programme in the absence of proliferation [10–12]. Transgenic animals expressing human AT_1Rs exclusively on cardiomyocytes further demonstrate that upregulation of AT_1Rs, in the absence of haemodynamic change, is sufficient to cause significant hypertrophy, and in at least one case, premature death due to cardiac failure [13, 14].

'Classical' AT_1R signalling, responsible for acute AngII actions such as vasoconstriction, is thought to occur via the G protein $G_{q/11}$, leading to activation of phospholipase C-β and the subsequent generation of second messengers diacylglycerol and inositol trisphosphate, which in turn stimulate protein kinase C (PKC) and mobilise intracellular calcium [15]. However, the hypertrophic effect of AngII in cardiac and non-cardiac cells is delayed (requires hours to days) and appears to involve the sequential and parallel activation of a variety of protein kinases that are normally associated with signalling pathways downstream of tyrosine kinase receptors. Kinases typically implicated in mediating cardiac growth include mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), the phosphatidylinositol 3-kinase (PI3K)-dependent signalling kinases, Akt/PKB and the mTOR/S6 kinase axis [12, 16, 17]. Activation of some of these pathways can be readily explained by 'classical' GPCR signalling, for example, PKC-dependent activation of ERK1/2. In contrast, mobilisation of additional growth-dependent kinases by the AT_1R, which lacks intrinsic tyrosine kinase activity, has been more difficult to elucidate. Axel Ullrich's group provided the first evidence for a GPCR-mediated 'transactivation' of the epidermal growth factor receptor (EGFR), potentially explaining 'non-classical' GPCR signalling outcomes related to proliferation [18]. Indeed, consistent with this paradigm, it now appears that AngII also 'hijacks' intracellular growth machinery by usurping EGFR signalling pathways to cause cardiac and smooth muscle hypertrophy [19–22].

GPCR transactivation paradigm

In 1996, Daub et al. established a pathway by which a variety of GPCR agonists could cause phosphorylation and activation of the plasma membrane-bound EGFR [18]. Furthermore, using the selective EGFR inhibitor tyrphostin AG1478 and a dominant-negative EGFR, the authors demonstrated that GPCR-mediated growth and proliferation were dependent upon EGFR 'transactivation'. Initially attributed to an intracellular signalling mechanism, the group subsequently demonstrated that EGFR transactivation also required matrix metalloprotease (MMP)-dependent extracellular cleavage of pro-heparin-binding EGF (HB-EGF), which liberated a soluble HB-EGF that could activate the EGFR (fig. 1) [23]. Accordingly, this 'triple membrane-passing signalling' (TMPS) paradigm has been verified for a variety of GPCRs in different cellular backgrounds (for reviews see [22, 24–28]). In particular, TMPS is pertinent to AngII-mediated cell proliferation and growth, including cardiac hypertrophy (table 1), although the exact mechanisms involved in MMP mobilisation and HB-EGF liberation are far from established. Given the critical function of the EGFR and its subtypes, matrix metalloproteases and HB-EGF in the developing and mature heart, it is reasonable to expect a predominant role of TMPS in AII-mediated cardiac hypertrophy.

EGFR transactivation in the heart

An important clue to the physiological function of the EGFR and its family members (HER2, HER3, HER4) in the myocardium came inadvertently from the anti-HER2 breast cancer drug, Herceptin, which caused dilated cardiomyopathy in a subset of patients [29]. Consistent with this, transgenic mice lacking HB-EGF [30], HER2 [31] or EGFR [32] in the heart developed a similar phenotype of cardiomyopathy, cardiac hypertrophy and premature death. One of the first direct demonstrations of AngII-mediated TMPS in the heart was provided by Thomas et al. (2002), who showed that AngII administration caused hypertrophy in primary cultures of neonatal cardiomyocytes in an MMP/EGFR-dependent manner [12]. Asakura et al. (2002) provided evidence for a functional role in vivo for the TMPS by demonstrating that ADAM12, a disintegrin MMP, was linked to the release of HB-EGF and subsequent EGFR transactivation in the intact heart following AT_1R and other GPCR stimulation [20].

The requirement for Gq in GPCR-mediated transactivation and the nature of the signalling molecules that link the receptor to EGFR transactivation are controversial [26, 27, 33, 34]. In the studies of Thomas et al. (2002), a Gαq inhibitory peptide prevented the induction of the hypertrophic marker, atrial natriuretic peptide, in accordance with the established hypertrophic effect of cardiac-specific overexpression of constitutively active Gαq [35–38]. In contrast, recent studies using G protein-uncoupled AT_1Rs indicate that EGFR transactivation may occur independent of Gαq, although it would appear that

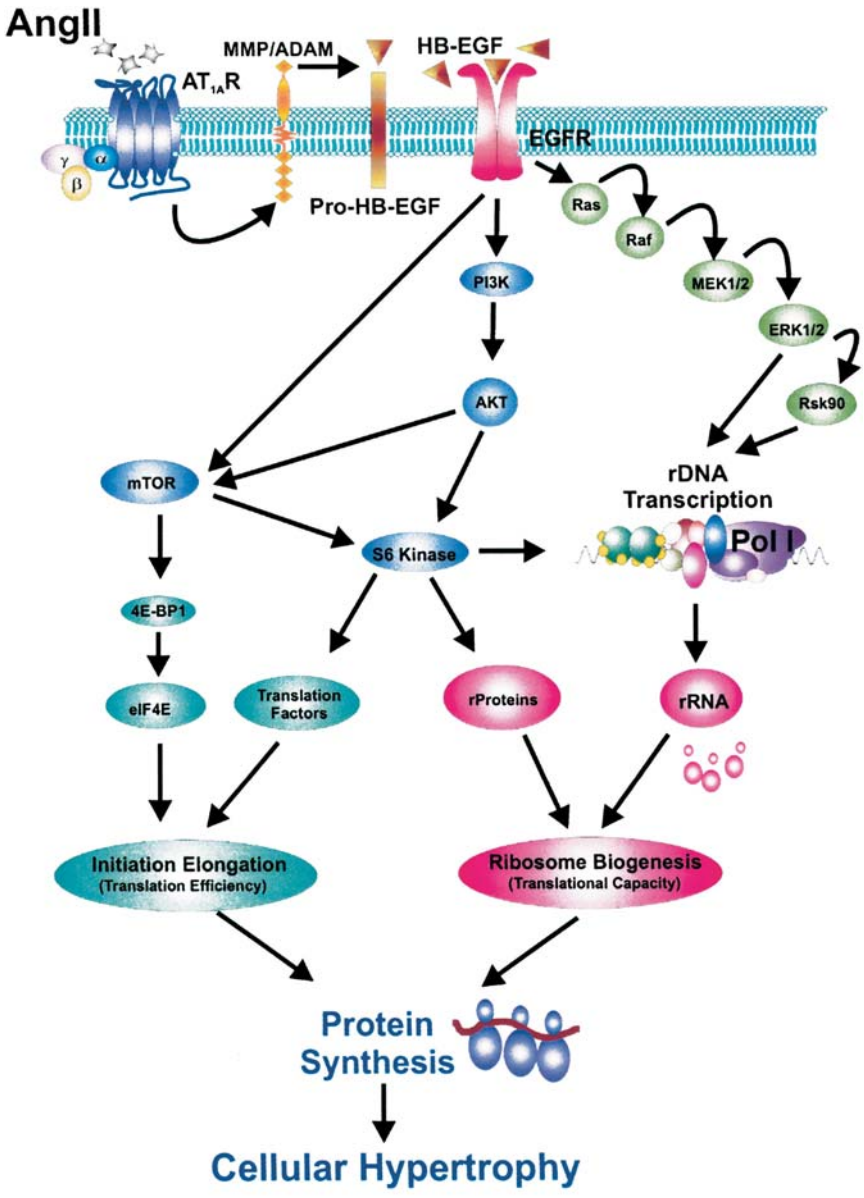

Figure 1. Schematic diagram depicting the proposed TMPS pathway by which angiotensin II stimulates cardiac hypertrophy. Current studies support a model whereby AT_1R-dependent transactivation of EGFRs on cardiomyocytes involves stimulation of membrane-bound metalloproteases, which in turn cleave EGFR ligands such as heparin-binding EGF from a plasma membrane-associated precursor. Subsequent activation of the EGFR leads to the mobilisation of signalling pathways thought to play pivotal roles in regulating cellular growth and proliferation, including ERK1/2, PI3K, Akt and mTOR/S6 kinase. The mechanisms by which the AT_1R activates the membrane-bound metalloproteases are unclear but may involve Ca^{2+}, Src and Pyk2.

further studies are required to definitively establish this in cardiomyocytes (refer to review by Thomas et al. within this issue) [39, 40]. Similarly, the role of downstream effectors of classical G protein-coupled AT_1R signalling in EGFR transactivation is also unclear. In the studies of Asakura et al., the ADAM12 responsible for the release of HB-EGF and subsequent EGFR transactivation was identified as a PKC-interacting molecule [20], whereas PKC and calcium inhibition failed to alter AngII-dependent EGFR activation, MAPK signalling or hypertrophy in isolated cardiomyocytes [12]. Current evidence supports a model whereby GPCR-mediated EGFR transactivation can occur via multiple mechanisms; the predominance of each mechanism most likely depends on the specific GPCR and cellular background involved, and perhaps the local extracellular environment. Evidently a certain amount of redundancy exists, since inhibition of one transactivation pathway can lead to unmasking of additional mechanisms within the one cell type [41].

Table I. AT_1R and EGFR transactivation.

Cell type	How transactivation was established	Ref.
Cardiac		
Rat neonatal cardiomyocytes infected with $AT_{1A}R$ adenovirus	AngII-mediated EGFR activation, ERK1/2 stimulation and cardiac hypertrophy were inhibited by AG1478 and MMP inhibitor, batimastat	[12]
In vivo and rat neonatal cardiomyocytes	Administration of HB-EGF MMP inhibitor, KB-R7785, attenuated AngII-induced LVH in mice and EGFR transactivation in vitro	[20]
Rat neonatal fibroblasts infected with AT_1R or Y319F adenovirus	AngII-mediated cell proliferation was abolished by AG1478 treatment or mutating the AT_1R to Y319F	[41]
Non-Cardiac		
COS-7	Y319F AT_1R mutant prevented EGFR phosphorylation and EGFR-dependent ERK1/2 activation, in comparison to wild-type AT_1R	[41]
Rat VSMCs in vivo and in vitro	AngII-mediated VSMC hypertrophy via ERK1/2, PI3K, Akt, p70S6K and translational repressor 4E-BP1 was attenuated by AG1478 or dominant-negative EGFR, HERCD533	[65]
Cultured rat VSMCs	AG1478 specifically inhibited AngII-dependent EGFR, ERK1/2 and Akt activation and DNA synthesis	[83]
Cultured human VSMCs	MMP inhibitors, batimastat and BB2116, a neutralising HB-EGF antibody and AG1478 inhibited MAPK activation	[84]
Mouse mesangial cells	AngII-induced protein synthesis was abolished by AG1478, a neutralising HB-EGF antibody and batimastat	[85]

Role of EGFR subtypes, ligands and MMPs

Elucidating the exact mechanism of AngII-mediated EGFR transactivation is complicated by the existence of four EGFR subtypes (table 2). In addition, the EGFR family of receptor tyrosine kinases are activated by trans-phosphorylation, and therefore require homo- or hetero-dimerisation within the family [42]. While all four EGFR subtypes are required for normal heart development, the exact contribution of the subtypes seems specific to distinct developmental stages of the heart, consistent with their ability to couple to different signalling pathways [43]. Their specific involvement in cardiac pathology associated with deregulation of the renin-angiotensin system in adult hearts and other cardiovascular tissues is less well defined, and forms part of ongoing studies by a number of laboratories.

HER2 is the preferred dimerisation partner for the other EGFR family members and can be transactivated via the EGFR by GPCR agonists, including AngII [18, 44]. In addition to its role in development, conditional deletion of HER2 in adult mice results in dilated cardiomyopathy, confirming the requirement for functional HER2 in the differentiated myocardium and providing a mechanism for the principal side effects (cardiomyopathy and heart failure) observed in patients undergoing chemotherapy with Herceptin [31]. HER3 is not believed to participate in cardiac function in adult animals, as messenger RNA (mRNA) and protein levels are undetectable after mid-embryogenesis [45]. In contrast, stimulation of HER4 can cause hypertrophy in both neonatal and adult cardiomyocytes, although its contribution to AngII-mediated LVH has not been investigated [45].

With the exception of HER2, which has no known high-affinity ligand, each EGFR subtype is activated by multiple polypeptide ligands of the EGF/neuregulin superfamily (table 2) [46]. Furthermore, the tissue-specific retention of membrane-bound precursors of many soluble EGFR ligands within the extracellular matrix, including HB-EGF, amphiregulin and betacellulin, provides an additional level of regulation for the TMPS [47]. To date, studies have focussed on the role of the HER1 EGFR subtype and HB-EGF in transactivation. Mice lacking HB-EGF exhibit significant valvulogenesis defects, a phenotype that is mimicked in EGFR null mice [30, 32]. Fur-

Table 2. EGFR subtypes and their ligands.

EGFR/ErbB1/HER1	HER2/ErbB2	HER3/ErbB3	HER4/ErbB4
EGF	no identified high-affinity ligand	neuregulin 1	HB-EGF
TGFα		neuregulin 2	betacellulin
Amphiregulin			epiregulin
HB-EGF			neuregulin 1
Betacellulin			neuregulin 2
Epiregulin			neuregulin 3
			neuregulin 4

thermore, inhibition of HB-EGF shedding blocks development of hypertrophy in response to pressure overload or GPCR agonists, including AngII, suggesting a major role for this ligand in mediating TMPS in adult mice [20]. Interestingly, stimulation of isolated cardiac myocytes with HB-EGF results in only modest cardiac hypertrophy compared to AngII (10 vs 35% increase in protein content, [W. G. Thomas, Lew and R. D. Hannan, unpublished observation]. These data suggest that while HB-EGF may be necessary for AngII-mediated hypertrophy, it is not sufficient for this process, and additional GPCR signalling is required to elicit maximal growth.

Like HB-EGF, members of the neuregulin family have been strongly implicated in the development of heart valves, cardiac conduction systems and growth repair/survival of adult myocytes [45, 48]. However, the specific contribution of this EGFR ligand family and others, such as betacellulin, to cardiac hypertrophy via AngII-dependent TMPS remains to be investigated.

Distinct sets of MMPs and a disintegrin and metalloprotease (ADAMs) are responsible for liberating soluble ligands from plasma membrane-associated precursors (proligands). MMP/ADAMs have been shown to mediate EGFR ligand release in a variety of settings, including the heart [12]. Furthermore, MMP inhibition has been demonstrated to regress or block the development of LVH and congestive heart failure in pigs [4, 49]. To date, more than six MMPs have been implicated in cardiac hypertrophy alone [50], and the identity of the protease that regulates HB-EGF shedding has received particular attention due to the recognised role of HB-EGF in GPCR-mediated transactivation. Several MMP and ADAM members have been proposed as the putative proHB-EGF convertase; however, the contribution of each MMP/ADAM to the TMPS appears to be highly reliant upon cellular background [47]. In the context of cardiac hypertrophy, ADAM12 has emerged as a likely regulator of HB-EGF shedding during GPCR- and pressure overload-mediated hypertrophy [20]. Unfortunately, however, genetic approaches to identify the exact set of MMP/ADAMs involved in specific GPCR-mediated events in the heart are complicated by their fundamental role in cardiac development, normal physiology and remodelling of the extracellular matrix during pathological states and possibly redundancy in function.

Finally, as well as activation of EGFRs by soluble ligands such as HB-EGF, evidence is accumulating to suggest that the membrane-associated, uncleaved forms of HB-EGF can activate EGFR in adjacent cells [51–54]. This 'juxtacrine' stimulation has been well characterised in kidney cells where membrane-associated HB-EGF, complexed with CD9, integrins and other factors, interacts with the EGFR on polarized epithelial cells. The extent to which this occurs between the various cell types in the heart has not been investigated.

AngII activation of EGFR via ROS

Reactive oxygen species (ROS) have been shown to modulate the activation state of EGFR family members [24, 55], and recent studies from non-cardiomyocytes have strongly implicated ROS/EGFR signalling in AngII-mediated growth and proliferation. For example, in vascular smooth muscle cells (VSMCs), AngII-stimulated ROS release leads to vascular hypertrophy via ERK1/2, PI3K/Akt and transcription initiation [15, 56, 57]. Importantly, AngII-mediated liberation of ROS, particularly H_2O_2, can lead to EGFR transactivation, where H_2O_2 acts upstream of the EGFR [58]. ROS can also induce HB-EGF mRNA upregulation [59], and Frank et al. (2003) recently demonstrated that GPCR-mediated ROS release could cause EGFR transactivation via the TMPS by stimulating MMP cleavage of HB-EGF [60]. Although few studies have directly examined the role of EGFR transactivation in AngII/ROS-mediated cardiomyocyte hypertrophy, circumstantial evidence suggests that it may be important. Chronic release of ROS can lead to LVH and heart failure progression [61, 62], while whole animal treatment with simvastatin, an HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor that prevents free radical formation, blocks LVH induced by chronic AngII infusion [63]. Furthermore, specific inhibition of the Ras/Raf/ERK1/2 pathway attenuated the effect of AngII-mediated ROS release in isolated cardiomyocyte hypertrophy [64]. Thus, given that ROS can stimulate MMP mobilisation and EGFR transactivation, appropriately targeted antioxidant therapy may be a viable option for treatment of AngII-mediated LVH.

Molecular signals that couple EGFR transactivation to hypertrophic growth

By utilising distinct pathways for classical signalling and growth, AII is able to impart its effects both qualitatively and temporally. Classical second messengers, such as inositol phosphates and calcium, respond to AT_1R activation within seconds. In contrast, hypertrophic growth requires de novo synthesis of protein, a process requiring hours to days. EGFR transactivation occurs within minutes, and thus provides an intermediate step between AngII stimulation and induction of cardiac hypertrophy [12, 18]. Auto-phosphorylation and trans-phosphorylation (by other kinases such as Src) of the c-terminal region of the EGFR provides specific docking sites for downstream intracellular signal transducers and adaptors that ultimately couple to physiological responses such as growth and proliferation. Activation of the Ras/Raf/ERK pathway is probably the best characterised; not only is it fundamental to EGF-mediated proliferation in fibroblasts, it is also essential for cardiomyocyte hypertrophy following AngII-

mediated transactivation of EGFR receptors [12, 19]. Similarly, other signalling pathways thought to play pivotal roles in coupling AngII to cellular growth and proliferation, including PI3K, AKT and mTOR/S6K, appear to be partially or totally dependent on EGFR transactivation [16, 17, 65]. Interestingly, early reports that AngII stimulation of cardiomyocytes activates STAT signalling [66, 67] might be explained by EGFR transactivation, since ligand-dependent phosphorylation of STATs by the EGFR has been well established in other cell types [68, 69].

A defining characteristic of hypertrophy is an elevation in global protein content without changes to DNA levels or cytokinesis [70]. A fact commonly overlooked is that increased ribosome biogenesis (i.e., an increase in translational capacity, fig. 1) is required for cardiomyocyte hypertrophy, as translational efficiency in contracting myocytes in vivo is already near-maximal [71]. The rate-limiting step in ribosome biogenesis is transcription of the ribosomal 45S gene (rDNA) by RNA polymerase I [72], suggesting that in cardiomyocytes, AT_1R-dependent signalling pathways subsequent to EGFR transactivation must converge on the rDNA transcription apparatus (fig. 1). In NIH3T3 fibroblasts, EGFR stimulation can activate rDNA transcription by direct ERK1/2- and/or p90 RSK-dependent phosphorylation of the rDNA transcription factors UBF and Rrn3 [73]. Similarly, activation of ribosomal protein S6 kinase (S6K), a kinase important for modulating protein synthesis via effects on translation of ribosomal proteins and translation initiation factors, has been shown to regulate rDNA transcription in NIH3T3 cells by modulating phosphorylation of the UBF carboxy-terminal activation domain [74]. Since EGFR signalling pathways shown to regulate UBF in NIH3T3 fibroblasts are also activated by AngII in an EGFR-dependent manner, both in cardiac and smooth muscle cells [16, 17], it is highly likely that the molecular mechanisms regulating ribosome biogenesis and cell growth in muscle cells upon stimulation are similar to those operating in fibroblasts following EGFR activation. Consistent with this, the cellular activity of UBF is critical for the regulation of rDNA transcription and protein synthesis during cardiac hypertrophy [75, 76] and is regulated by ERK1/2 and S6K during AngII stimulation of cardiomyocytes [R. D. Hannan and Y. Brandenburger, unpublished data].

Clinical implications of TMPS

One unpredicted but potentially desirable consequence of dual classical EGFR-independent and non-classical, EGFR-dependent GPCR signalling in the heart is the prospect of selective inhibitors that can distinguish between the two signalling paradigms. Thus, it may be possible to develop small molecule inhibitors that block the EGFR-dependent growth effects of chronic GPCR activation, without interfering with other essential and acute GPCR-dependent events such as vasoconstriction and contraction. Although the precise mechanisms of AngII-mediated EGFR transactivation and subsequent cardiac hypertrophy remain to be fully elucidated, a number of potential therapeutic targets can be inferred from the TMPS paradigm.

GPCR activation state

The requirement of G protein coupling for EGFR transactivation remains controversial. However, cardiac-specific overexpression of a constitutively active Gq subunit and G protein inhibition suggests that the coupling state of the receptor may influence TMPS (discussed in more detail in a companion review in this issue) [35–38, 77]. As such, development of small molecule drugs that preclude the AT_1R from entering a conformation that preferentially promotes TMPS signalling may prevent the development of LVH. Conversely, Seta et al. (2003) have proposed a G protein-independent mechanism of EGFR transactivation whereby a single tyrosine mutation (Y319F) in the AT_1R carboxyl tail is sufficient to obliterate TMPS [41]. The authors suggest that this mutation disrupts a physical association between the AT_1R and EGFR, providing an attractive drug target.

MMP/ADAM activation

As indicated above, it is likely that EGFR transactivation is intricately regulated by the identity of the activated MMP/ADAM. As such, a possible therapeutic target for TMPS-mediated cardiac hypertrophy might be the MMP/ADAM required for liberation of hypertrophic EGFR ligands. Identification of the MMP/ADAM(s) involved will most likely necessitate high-throughput screening of substrates using RNA interference (RNAi) approaches given the number of MMP/ADAMs already identified. Some caution is required, however, due to the dependence on cellular background and signalling pathway for each MMP/ADAM. An MMP found to mediate LVH in rats might have no therapeutic utility in humans. Furthermore, as described above, inhibition of MMP signalling may have undesirable effects on normal cardiac function and homeostasis.

EGFR subtypes and EGFR ligands

Finally, identification of the dimerisation partners and interacting ligands would facilitate the design of inhibitors that could target the specific EGFR family interactions required for hypertrophy. Inhibitors that bind at the interface for dimerisation or trans-phosphorylation would potentially reduce non-specific interactions and side effects. Indeed, some EGFR antagonists already prevent dimeri-

sation as a consequence of their tyrosine kinase inhibition [78, 79]. Thus far, HB-EGF is the only EGFR ligand implicated in cardiac hypertrophy via the TMPS, and inhibitors have already been identified that prevent HB-EGF liberation and subsequent LVH [20]. However, HB-EGF also has important physiological functions, including wound healing and renal repair, suggesting that such an approach would only be of use if the drug is targeted specifically to the heart [80, 81].

Conclusions and future directions

In recent years, our perception of GPCR modulation of cell growth has expanded considerably, from mechanisms based on simple G protein coupling and established second messengers to more complex processes involving shedding of HB-EGF and transactivation of the EGFR. Multiple EGF ligands and receptors and an apparent redundancy in the enzymes responsible for shedding EGF ligands compound this complexity. The reason why such elaborate systems have evolved is not clear, but it may explain why GPCRs, like the AT_1R, have chosen to 'hijack' these receptors and their signalling pathways for proliferative and hypertrophic growth. Usurping of EGFRs also potentially allows GPCRs to separate acute, homeostatic actions from the commitment to increase ribosomal biogenesis and promote cell growth. EGFR transactivation may not be limited to GPCR-mediated hypertrophy but have a broader role in the regulation of cardiac growth in response to a variety of stimuli. For example, substantial evidence has accumulated to demonstrate that mechanical stretch is associated with transactivation of the EGFR and, in some cases, growth. This may provide an explanation for why transgenic mice lacking the AT_{1A} receptor still developed LVH after mechanical stretch [82].

Clearly, future research must focus on the mechanism of EGFR transactivation by AT_1Rs in the heart. Such studies should encompass issues including the requirement or otherwise for G protein activation, the means by which specific MMPs and ADAMs are activated and regulated, and the identification of the precise EGFR-related signals that impinge on the protein synthetic apparatus controlling cell growth. Finally, deeper understanding of the contribution of EGFR transactivation to AT_1R-mediated cardiac hypertrophy may provide new avenues for selectively antagonising this response in relation to cardiovascular dysfunction.

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