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RESEARCH HIGHLIGHT

Self-made allostery: endogenous COMP antagonizes pathologic $AT1_AR$ signaling

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The identification and development of biased modulators of proximal G protein-coupled receptor (GPCR) signaling to prevent pathological disease progression has been at the forefront of recent drug discovery efforts. Recently, Fu et al. identified cartilage oligomeric matrix protein (COMP) as an endogenous negative allosteric modulator of β-arrestin (βarr)-dependent angiotensin II (Ang II) Type 1A receptor ($AT1_AR$) signaling, which attenuates the development of abdominal aortic aneurysm (AAA), a chronic inflammatory vascular disease lacking pharmacological treatment options.

There is now wide recognition that GPCRs exist within a spectrum of structures and that their ligands act to stabilize certain conformations to engage distinct proximal transducers.[1] Classically, orthosteric GPCR agonists induce conformations of their cognate receptors to elicit both G protein activation, to induce a rapid signaling response, and GPCR kinase (GRK)-dependent recruitment of the ubiquitous scaffolding proteins βarr1 and βarr2, which act to desensitize the receptor and/or maintain additional signaling responses.[2] However, compared to a reference "gold standard" agonist, such as a natural endogenous agonist,[3] some GPCR ligands more strongly promote G protein activation versus βarr recruitment, or vice versa. The relative ability of a ligand to elicit a stronger activation of one signaling response over another has been termed biased agonism. Additionally, endogenous allosteric modulators of GPCRs have been identified in recent years to be able to impart negative or positive signaling bias by changing the effect of the natural ligand on its GPCR structure.[4] As studies have increasingly shown that biased modulation of GPCRs can relay differential outcomes in a cell-specific manner, harnessing this property to promote beneficial, and prevent pathologic, outcomes during disease progression has become a therapeutic goal of recent drug discovery efforts.

$AT1_AR$ in particular has been explored for many years in relation to the ability of designer orthosteric ligands to induce signal bias in comparison to Ang II. $AT1_AR$ is widely expressed throughout the cardiovascular system and regulates cardiac, vascular and renal effects, chronic activation of which can contribute to the development of numerous cardiovascular pathologies.[5] Thus far, application of biased $AT1_AR$ ligand pharmacology to a pathologic condition has centered on the ability of Ang II-derived peptides to promote βarr-dependent cardiac contractility in the absence of maladaptive Gq protein-dependent remodeling during heart failure (reviewed in[2]). Mechanistically, recent studies using protein crystallography, advanced spectroscopy and molecular simulations of $AT1_AR$ with modified peptide ligands have begun to define how these orthosteric agonists induce biased signaling at a structural level, revealing combinations of transmembrane domain movements associated with differing degrees of a fully activated Gq protein-bound state and an occluded state allowing βarr coupling (reviewed in[1]). Since $AT1_AR$ biased ligand discovery efforts have mainly centered on synthetic orthosteric ligands, whether endogenous biased allosteric modulators of $AT1_AR$ exist and impart pathophysiologically-relevant outcomes on Ang II-mediated signaling has been unclear.

Publishing recently in *Cell Research*, Fu et al. have identified COMP as an endogenous negative allosteric modulator of βarr-dependent $AT1_AR$ signaling that dampens AAA incidence.[6] This is an exciting study for many reasons, including that local expression and secretion of COMP within blood vessels themselves would allow it to mediate vascular homeostasis and dampen AAA development via regulation of various $AT1_AR$-expressing cell types (Fig. 1). This would be consistent with previous reports demonstrating that although global $AT1_AR$ deletion protects against AAA in a mouse model of AAA, neither endothelial cell- nor vascular smooth muscle cell (VMSC)-specific $AT1_AR$ deletion was sufficient to do so,[7] and that monocyte-expressing $AT1_AR$ contributes to the development of AAA.[8] Translationally, the authors report that a decrease in plasma COMP level is negatively associated with human AAA, which was also observed more specifically in the suprarenal aortas of chronic Ang II-infused $ApoE^{-/-}$ mice, a common AAA model. Further, the authors demonstrate that COMP deficiency ($COMP^{-/-}$) even on a wild-type background (C57BL/6) leads to increased incidence of AAA in response to Ang II infusion; however, crossing $COMP^{-/-}$ mice with $AT1_AR^{-/-}$ mice, or overexpressing COMP either generally within the suprarenal aorta or specifically in VMSC, protects against AAA.[6]

To understand the mechanism by which COMP acts at $AT1_AR$ to oppose AAA development, Fu et al. performed an extensive series of molecular assays and found that COMP directly binds to the N-terminus of $AT1_AR$, which neither decreases Ang II binding to its orthosteric site nor modulates Gq protein activation or downstream signaling.[6] Rather, COMP acts to attenuate the ability of $AT1_AR$ to engage its high-affinity βarr-binding conformation in response to Ang II, thereby decreasing βarr recruitment and its associated functions including $AT1_AR$ internalization and desensitization. Thus, COMP acts as a negative allosteric modulator of βarr-dependent $AT1_AR$ signaling; however, the conformational changes of $AT1_AR$ induced by COMP to relay this effect remain unclear. Based on the structural changes that have been reported to occur within $AT1_AR$ in response to modified orthosteric ligands,[1] COMP presumably promotes the distribution of Ang II-occupied $AT1_AR$ toward its fully active Gq protein-coupled state

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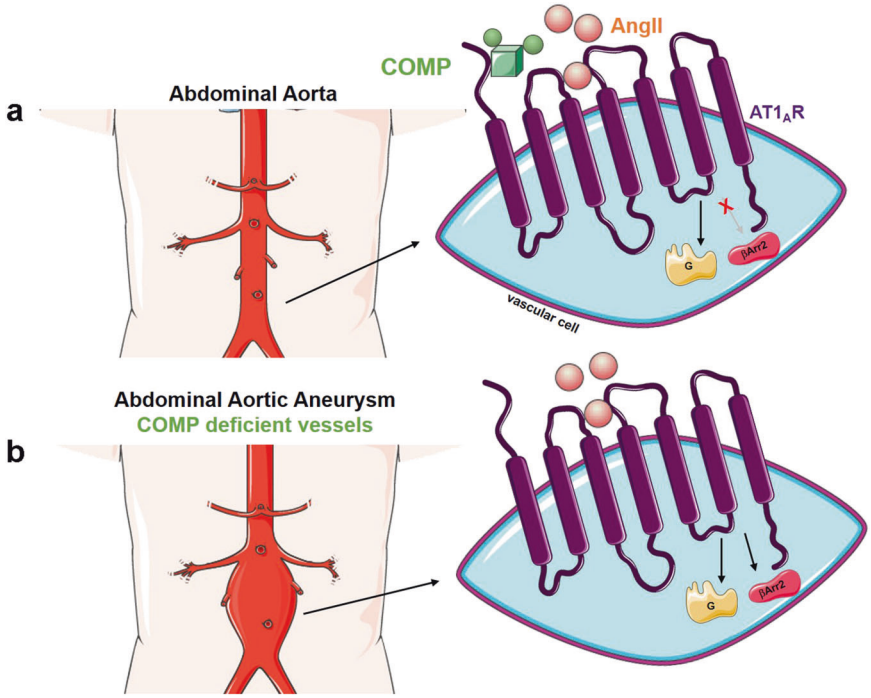

Fig. 1 COMP, an endogenous negative allosteric modulator of βarr-dependent $AT1_AR$ signaling, offsets AAA development. a Within the abdominal aorta, COMP directly binds $AT1_AR$ at its N-terminus to allosterically antagonize pathological βarr2-dependent signaling. **b** During the development of AAA, COMP levels decrease, relieving the inhibition of βarr-dependent $AT1_AR$ pro-inflammatory signaling.

and away from its transducer-occluded state, thereby reducing βarr coupling. Regardless of the structural effect of COMP at the level of the receptor, its negative regulatory impact on $AT1_AR$-mediated βarr signaling fits well with the previously reported observation that βarr2 deficiency attenuated AAA formation.[9] In that study, βarr2 deletion was associated with reduced aortic inflammation and remodeling responses to Ang II infusion, effects consistent with the enhanced aortic inflammatory readouts in $COMP^{-/-}$ mice observed by Fu et al. that were reversed by crossing $COMP^{-/-}$ and $βarr2^{-/-}$ mice,[6] and consistent with known βarr2-dependent effects on the regulation of inflammatory signaling.[10]

Finally, to advance their findings toward a therapeutic strategy, Fu et al. identified one of four different EGF repeat motifs within COMP, specifically EGF2, that directly interacts with the N-terminus of $AT1_AR$ and is required for COMP interaction with the receptor,[6] orienting the C-terminus of COMP toward the first extracellular loop (ECL1) of $AT1_AR$. Impressively, suprarenal aortic overexpression of a peptide encoding EGF2 was sufficient to decrease Ang II-induced AAA incidence in the $ApoE^{-/-}$ mouse model. This suggests that the orientation of C-terminal COMP with ECL1 of $AT1_AR$ is dispensable for the negative allosteric regulation of the receptor and that the interaction between EGF2 and the N-terminus of $AT1_AR$ alone may induce the required conformational changes to antagonize Ang II-mediated βarr recruitment. Thus, a pharmacologic strategy to allosterically modulate the N-terminus of $AT1_AR$ may offer a new approach to attenuate AAA incidence via biased antagonism of βarr-dependent aortic inflammation.

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