Angiotensin II mediates cardiomyocyte growth signalling *via* ErbB4

H. Chan,[1,2] N.J. Smith,[1] A. Agrotis,[1] R.D. Hannan[3] and W.G. Thomas ,[1] [1]Baker Heart Research Institute, Melbourne, VIC 8008, Australia, [2]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC 3800, Australia and [3]Peter MacCallum Cancer Centre, East Melbourne, VIC 3002, Australia.

Cardiomyocyte hypertrophy induced by angiotensin II (AngII) is mediated by the type 1 AngII receptor (AT_1R), a G protein-coupled receptor, which is targeted clinically to alleviate hypertension and the complications of cardiovascular disease. At the cellular level, activated AT_1Rs promote growth of renal, vascular and cardiac tissues by *transactivating* epidermal growth factor receptors (EGFRs), presumably via shedding of EGF-like ligands and the activation of one or more EGFR family members. Four structurally-conserved EGFR receptor tyrosine kinases exist, and these are termed ErbB1-4. Screening a panel of EGF-like ligands for their capacity to induce cardiomyocyte hypertrophy (measured by changes in protein:DNA ratio of isolated neonatal cardiomyocytes) revealed that betacellulin, neuregulin1-β1 and neuregulin2β (which predominantly act through ErbB4) were the most potent activators (147.9 ± 1.6, 134.6 ± 2.0, and 133.7 ± 2.1% compared to control, respectively). No small molecule selective inhibitors of ErbB4 are available, so we have developed small interfering RNAs that target ErbB4 gene expression (ErbB4 siR) to allow examination of the requirement for ErbB4 in cardiomyocyte hypertrophy. Interestingly, four major splice variants of ErbB4 exist and we used RT-PCR to show that these are all expressed in rat cardiomyocytes as well as whole human heart. Hence, we designed our siR to target a common region of the ErbB4 mRNA sequence and then confirmed efficient knockdown in HEK293 cells transiently expressing the separate ErbB4 isoforms. Using promoter-driven luciferase constructs for genes related to cardiomyocyte growth (ANP, MLC2V and cyclinD), we observed that *pan*knockdown of ErbB4 prevented the ability of neurregulin1/AngII to selectively activate ANP, CycD and MLC2V. This decrease was reversed by co-expression of specific ErbB4 isoforms mutated to make them resistant to siR knockdown, confirming that ErbB4 appears to mediate AngII-dependent cardiomyocyte hypertrophy and that this is mediated in an isoform-specific manner.