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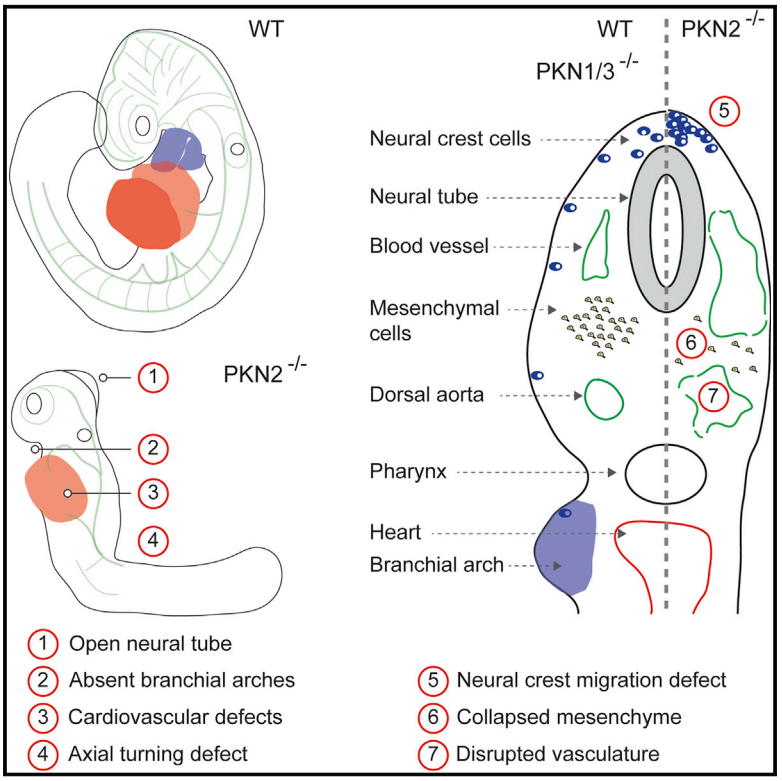
Knockout of the PKN Family of Rho Effector Kinases Reveals a Non-redundant Role for PKN2 in Developmental Mesoderm Expansion

Graphical Abstract



Highlights

- PKN2, but not PKN1 or PKN3, is essential during mouse embryogenesis
- PKN2 knockout causes severe cardiovascular and morphogenetic abnormalities
- PKN2 is required for mesenchymal growth and neural crest migration in vivo

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In Brief

The Rho effector PKN kinases regulate diverse cellular functions, but their in vivo function is unexplored. By systematically targeting the PKN family, Quetier et al. reveal a unique role for PKN2 during developmental growth and morphogenesis. These findings impact on developmental disorders and the targeting of PKN in disease.

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Knockout of the PKN Family of Rho Effector Kinases Reveals a Non-redundant Role for PKN2 in Developmental Mesoderm Expansion

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SUMMARY

In animals, the protein kinase C (PKC) family has expanded into diversely regulated subgroups, including the Rho family-responsive PKN kinases. Here, we describe knockouts of all three mouse PKN isoforms and reveal that PKN2 loss results in lethality at embryonic day 10 (E10), with associated cardiovascular and morphogenetic defects. The cardiovascular phenotype was not recapitulated by conditional deletion of PKN2 in endothelial cells or the developing heart. In contrast, inducible systemic deletion of PKN2 after E7 provoked collapse of the embryonic mesoderm. Furthermore, mouse embryonic fibroblasts, which arise from the embryonic mesoderm, depend on PKN2 for proliferation and motility. These cellular defects are reflected in vivo as dependence on PKN2 for mesoderm proliferation and neural crest migration. We conclude that failure of the mesoderm to expand in the absence of PKN2 compromises cardiovascular integrity and development, resulting in lethality.

INTRODUCTION

The Rho family guanosine triphosphatases (GTPases), which includes Rho, Rac, and Cdc42, are regulators of cell shape, adhesion, and motility and as such are critical in development (Bustelo et al., 2007). Numerous studies have examined the roles of Rho family members at a biochemical and cellular level; however, their ubiquitous expression and pleiotropic roles have made elucidation of in vivo function difficult. This has in part been addressed using tissue-specific conditional alleles of Rho family members (D'Amico et al., 2009). To gain a deeper insight, it is necessary to examine the roles of specific effector pathways. Among the diverse array of effectors are many Ser/Thr kinases, which include the Rho family-activated kinases (ROCK1/2), p21-activated kinases (PAK1–PAK6), and protein kinase N (PKN) kinases (Zhao and Manser, 2005).

The PKN kinases represent a subfamily of the protein kinase C (PKC) family, sharing a high degree of homology within their C-terminal kinase domains (Mukai, 2003). The divergent N-terminal regulatory domains bear a C2 domain and three polybasic coiled-coil HR1 domains, which confer binding and regulation by Rho family members. While other mammalian PKCs lack HR1 domains and Rho responsiveness, PKC orthologs in yeast bear two HR1 domains and signal downstream of Rho1; yeast PKC also retains the regulatory C1 and C2 domains found in mammalian PKC isoforms, suggesting an evolutionary split between the Rho-responsive and the Rho-independent functions of the PKC family. There are three PKN family members in mammals (PKN1–PKN3, also known as PRKs), which vary in their regulation. While both PKN1 and PKN2 are activated by RhoA and Rac1 (Flynn et al., 1998; Vincent and Settleman, 1997), they respond differently to phosphoinositides and fatty acids (Mukai, 2003). PKN isoforms also respond to distinct stimuli and bind to distinct effectors, highlighting functional divergence within the family. Expression patterns also differ; PKN1 and PKN2 mRNAs are ubiquitous in expression, while PKN3 is more restricted to muscle, liver, and endothelial cells (Aleku et al., 2008; Palmer et al., 1995). In vivo, a role for PKN1 in germinal center formation has been reported (Yasui et al., 2012).