Pcgf5 Contributes to PRC1 (Polycomb Repressive Complex 1) in Developing Cardiac Cells

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Abstract

Polycomb-group (PcG) proteins maintain transcriptional silencing through specific histone modification and are essential for cell-fate transition and proper development of embryonic and adult stem cells. Recent advances in molecular analysis of PcG proteins have revealed that the distinct subunit composition of PRC1 confers specific and nonoverlapping functions for regulation of embryonic and adult stem cells. Here, we provide an overview of recent findings regarding the role of PcG proteins in cardiac development, with focus on the diversity of PcG complexes.

Keywords

Polycomb-group protein • Cardiac development • Transcriptional silencing • Histone modification

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43.1 Introduction

Cardiac development is a complex and ordered process that requires cellular specification, proliferation, and differentiation, as well as further migration of cell populations from diverse sites. The primary heart field (PHF) originates in the anterior splanchnic mesoderm, then gives rise first to the cardiac crescent, later to the linear heart tube, and ultimately contributes to parts of the left ventricular (LV) region. The second cardiogenic region, known as the second heart field (SHF), lies in the anterior, posterior, and dorsal to the linear heart tube and is derived from the pharyngeal mesoderm located medial and anterior to the cardiac crescent. Cells from the SHF are added to the developing heart tube and give rise to the outflow tract (OFT), right ventricular (RV) region, and main parts of atrial tissues [1].

Congenital heart defects (CHDs) represent the most common anomaly seen in human newborns, with a prevalence of approximately 1 % of all births [1]. Traditionally, focus on causes of CHD has involved transcriptional networks during cardiogenesis, because correct alignment and septation of cardiac structures regulated by cardiac specific transcriptional factors, such as *Tbx1*, *Tbx5*, *Tbx20*, *Gata4*, and *Nkx2-5*, are essential for cardiac morphogenesis [1]. In addition to these multiple genetic factors, recent studies have shown that some chromatin remodeling factors moderate gene expression to control cardiogenesis and are also involved in the molecular pathogenesis of CHD [2–7].

Polycomb-group (PcG) proteins maintain transcriptional silencing by regulating chromatin configuration [8, 9]. There are two principal PcG repressive complexes (PRCs), PRC1 and PRC2. Mammalian PRC2 contains four core proteins (Ezh1/2, Eed, Suz12, Rbbp4/7) and trimethylates histone H3 at lysine 23 (H3K27), while the other complex, PRC1, consists of a combination of several protein families, including chromobox (Cbx), Ring, polyhomeotic (Ph), and posterior sex combs (Psc), and induces mono-ubiquitination of histone H2A at lysine 119 (Fig. 43.1). In recent studies, PRC1 have been divided to Cbx-PRC1 (canonical PRC1) and Rybp-PRC1 (noncanonical PRC1) [10, 11]. Since development of high-throughput techniques for analyzing the genome in the past decade, PcG-mediated transcriptional repression has resulted in increased molecular information regarding its role in a number of important biological activities such as cell cycle progression, differentiation, and cell-fate transition in multiple cell types and tissue contexts, including embryonic, adult, and cancer stem cells. This PcG-mediated transcriptional repression can vary during development and among cell types. However, the precise role in the context of cell conditions remains unclear.

43.2 PcG Functions in Cardiac Development

In this review, we primarily focused on the functions of PcG proteins in cardiac development. Their roles have been elucidated via generation of knockout (KO) mice for each of the PcG components. Among the PRC2 components, loss

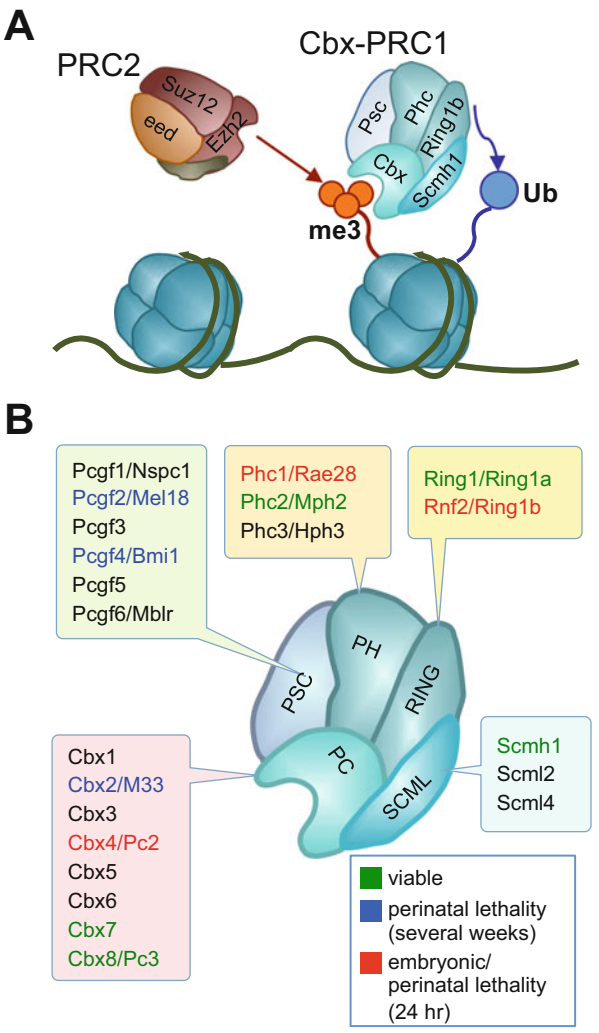

Fig. 43.1 PcG complexes in mammals. (**A**) Molecular functions of PcG complexes (PRCs). PRC2 trimethylates lysine 27 of histone H3. Canonical PRC1 binds to the H3K27me3 mark and mediates the mono-ubiquitination of histone H2A at lysine 119. (**B**) Canonical PRC1 components and results of KO of each in mice

of Suz12, Ezh2, or Eed results in embryonic lethality during the early postimplantation stage [12–14]. To address the role of PRC2 in cardiac development, Ezh2 and Eed were conditionally inactivated in specified cardiac cells using Nkx2-5:Cre or TnT:Cre [3, 4]. Inactivation of Ezh2 by Nkx2-5:Cre ($Ezh2^{NK}$) and Eed by TnT:Cre (Eed^{TnT}) led to embryonic lethality and several cardiac defects including compact myocardial hypoplasia, whereas inactivation of Ezh2 by TnT:Cre ($Ezh2^{TnT}$) did not result in severe defects in cardiogenesis despite a modest upregulation of some cardiac genes, probably because of the redundant functions of Ezh1 and Ezh2.

Embryos deficient of *Ring1b*, a core component of PRC1, also displayed early embryonic lethality caused by gastrulation arrest [15]. Although early developmental arrest in *Ring1b* KO embryos was partially restored by inactivation of Cdkn2a

(Ink4a/ARF), cardiac tissue did not develop in double-KO embryos. Unlike early developmental defects seen in KO mice lacking some of the core PRC1 and PRC2 components, deficiency of other components has been shown to give rise to restricted effects. For example, loss of Rae28/Phc1 resulted in perinatal lethality with cardiac anomalies, double outlet right ventricle, and tetralogy of Fallot [6, 7]. In addition to cardiac defects, *Rae28/Phc1* deficient mice also showed craniofacial developmental defects, as well as thymus and parathyroid gland defects as seen in human DiGeorge syndrome.

Among Cbx proteins, Cbx4 may play an important role in cardiogenesis. SUMO-specific protease 2 (SENP2) was reported to regulate transcription of *Gata4* and *Gata6*, mainly through alteration of the occupancy of Cbx4 on their promoters [5]. In *SENP2*-deficient embryos, sumoylated Cbx4 accumulates on the promoters of target genes, leading to transcriptional repression of *Gata4* and *Gata6*. Furthermore, *Cbx4* mutant mice displayed postnatal lethality with severe hypoplasia of the developing thymus as a result of reduced thymocyte proliferation. However, the function of Cbx4 in cardiogenesis has not been clearly elucidated [16]. Thus, PcG proteins are essential for molecular regulation of the expression of several cardiac genes during embryogenesis and important for cardiac morphogenesis.

43.3 Diversity of PcG Proteins

In our recent studies, expression of the PRC1 components Ring1b, Bmi1/Pcgf4, and Rae28/Phc1 was detected in embryonic hearts containing both the PHF and SHF embryonic cardiac fields (Fig. 43.2), though their expression patterns were not restricted in cardiac cells. Despite the ubiquitous expression of PRC1 components, mice with single PcG KO except for the core proteins show more distinct and restricted phenotypes. Among Psc proteins, genetic deletion of *Mel18/Pcgf2* or *Bmi1/Pcgf4* resulted in postnatal lethality caused defects in anterior-posterior specification, while there was no effect on cardiogenesis [17, 18]. Also, *Mel18/Bmi1* double-KO mice died around E9.5 and exhibited more severe developmental defects than those with KO of either alone, suggesting that Mel18/Pcgf2 and Bmi1/Pcgf4 have partially redundant functions [18]. In addition to functional redundancy, recent studies have shown that distinct Cbx and Pcgf proteins confer specific and nonoverlapping functions of PRC1 in embryonic and adult stem cells [10, 11]. Exchanging Cbx protein in canonical PRC1 is involved in the switch from self-renewal to a differentiation state, whereas Pcgf and other noncanonical components, such as Rybp, Kdm2b, and L3mbtl2, also confer restricted functions to PRC1.

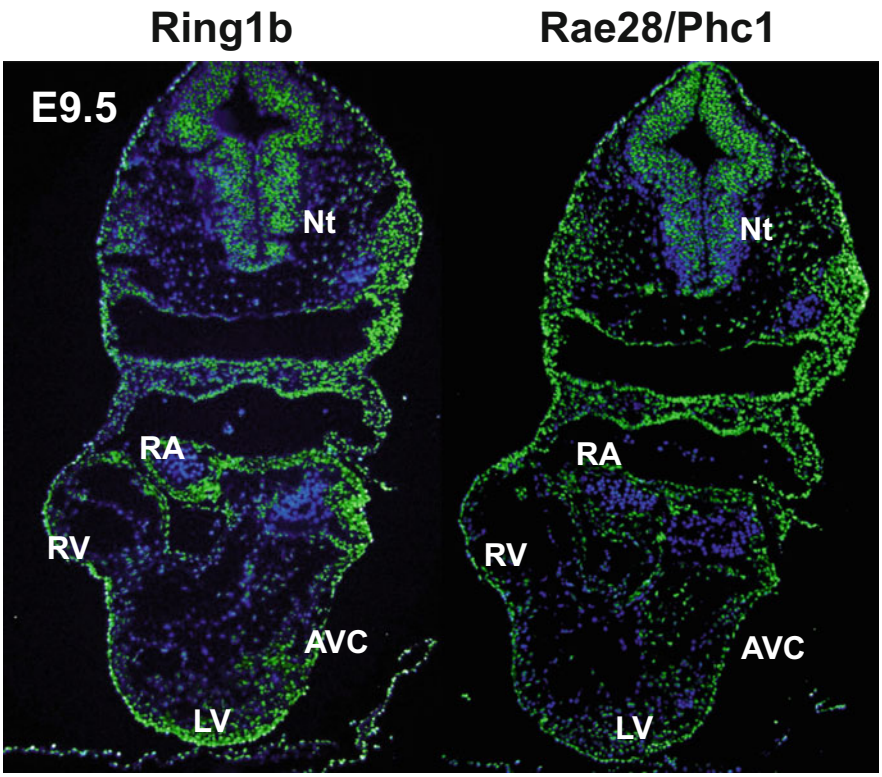

Fig. 43.2 Expression patterns of Ring1b and Rae28/Phc1 in E9.5 mouse embryos. Anti-Ring1b and anti-Rae28/Phc1 antibody-positive cells are ubiquitously distributed on E9.5. AVC, atrioventricular canal, LV, left ventricle, Nt, neural tube, RA, right atrium, RV, right ventricle

43.4 Pcgf5 Expression in the Developing Heart

Recently, we identified *Pcgf5* as an upregulated gene during cardiomyocyte differentiation of ES cells as well as strong expression of *Pcgf5* in cardiac fields during the early embryonic stages as compared to other embryonic tissues [19] (Fig. 43.3). Unlike *Bmi1*/*Pcgf4*, *Pcgf5* expression, patterns were more restricted to early mouse embryos. Our hypothesis states that exchanging Pcgf components within PRC1 determines the specificity of their function in the developing heart.

43.5 Conclusions

Recent increasing evidence obtained in experiments with embryonic and adult stem cells indicates that the diversity of PRC components, particularly PRC1, contributes to specification of their function (Fig. 43.4). Furthermore, transcriptional repression

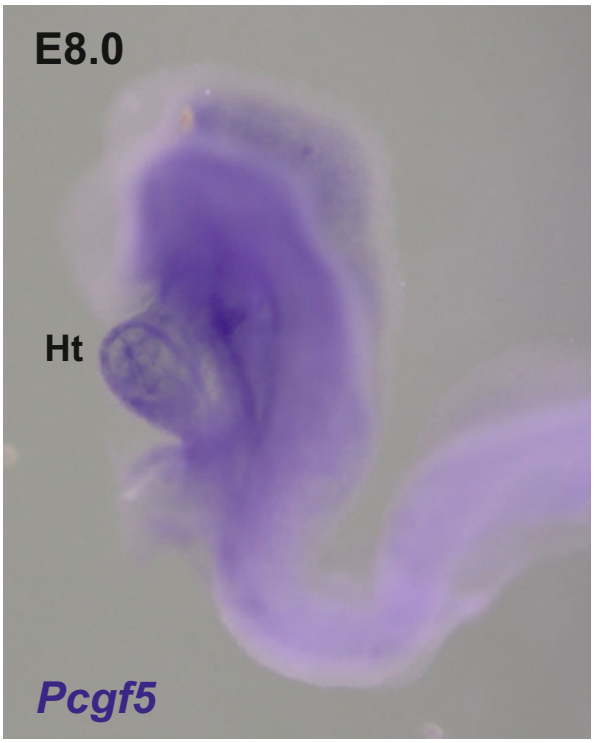

Fig. 43.3 Expression pattern of *Pcgf5* in E8.0 mouse embryos. Whole-mount in situ hybridization (WISH) showing expression of *Pcgf5* at E8.0. High Pcgf5 expression was observed in developing hearts. Ht, heart

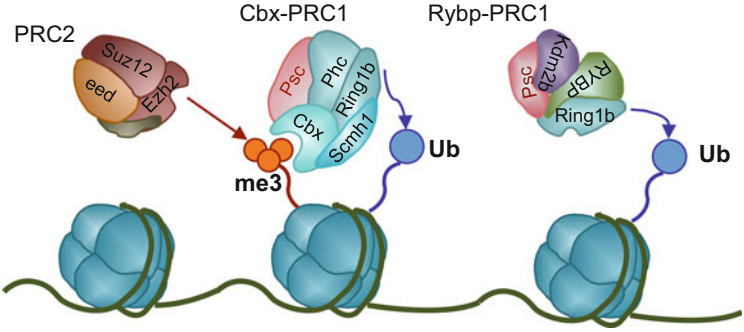

Fig. 43.4 Recent insight regarding PRC1 complexes. Canonical PRC1 (Cbx-PRC1) is recruited to H3K27me3 and causes mono-ubiquitination of lysine 119 of histone H2A. Noncanonical PRC1 (Rybp-PRC1) mediates the mono-ubiquitination of H2A in a PRC2-independent manner

by PcG is essential for cardiogenesis. Understanding PcG functions and more detailed characterization of the PcG complex during cardiogenesis may be crucial for elucidating the molecular mechanisms regulating cardiac development.

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