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Wnt2 is a direct downstream target of GATA6 during early cardiogenesis

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Abstract

The GATA4, 5 and 6 subfamily of transcription factors are potent transactivators of transcription expressed within the precardiac mesoderm. However, little is known of the immediate downstream targets of GATA-factor regulation during the earliest stages of cardiogenesis. Using the P19-CL6 embryonal carcinoma (EC) cell line as an in vitro model of cardiogenesis, we show that GATA6 is the most abundantly expressed of the GATA factors in presumptive cardiac cells. Consequently, we performed a microarray screen comparing mRNA from control EC cells, early in the cardiac differentiation pathway, with those in which GATA6 had been overexpressed. These studies identified 103 genes whose expression changed significantly and this was verified in a representative array of these genes by real-time RT-PCR. We show that early cardiac expression of one of these genes, Wnt2, mirrors that of GATA6 in vitro and in vivo. In addition, its upregulation by GATA6 in differentiating EC cells is mediated by the direct binding of GATA-factor(s) to the cognate Wnt2 promoter, suggesting Wnt2 is an immediate downstream target of GATA-factor regulation during early cardiogenesis.

Keywords: Cardiogenesis; GATA; Heart; Target; Wnt2

1. Introduction

The molecular basis of vertebrate cardiogenesis is increasingly becoming understood. The formation of a multi-chambered, beating heart requires the activation of a cascade of transcription factors under tight temporal and spatial control and congenital heart defects can result if this ordered arrangement is perturbed (for review see (Bruneau, 2002)). Many of these regulatory factors have been identified and cloned and it has emerged that the GATA4, 5 and 6 sub-family of transcription factors, which display partially overlapping expression domains in heart and some endodermally derived tissues play an integral role in cardiogenesis (for reviews see (Molkentin, 2000; Patient and Mcghee, 2002; Peterkin et al., 2005)). Studies of *cis*-regulatory elements have demonstrated important roles of GATA factors in promoting the expression of many myocardial genes, including α- and β-myosin heavy chain (α- and β-MHC), cardiac troponin C, atrial natriuretic peptide (ANF) and brain natriuretic peptide (BNP) (Charron and Nemer, 1999; Charron et al., 1999). GATA4, 5 and 6 are co-expressed within the prospective cardiac mesoderm from the earliest time of its specification (Charron and Nemer, 1999; Peterkin et al., 2005). However, in the mouse, the expression pattern of GATA5 subsequently diverges from that of GATA4 and 6: GATA5 becoming restricted to the endocardium (Morrisey et al., 1997), whereas GATA4 and 6 are expressed within the myocardium throughout development and adult life (Arceci et al., 1993; Kelley et al., 1993; Heikinheimo et al., 1994).

Given the high degree of homology within the DNA binding domains of GATA-family members (Laverriere et al., 1994; Jiang and Evans, 1996), a degree of functional redundancy has been suggested within domains in which different family members are co-expressed. This is particularly relevant in the case of GATA4 and 6 in the developing heart, where the expression patterns overlap both temporally and spatially.

Targeted disruption of GATA4 or 6 expression in the mouse, however, resulted in very different phenotypes, both of

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which are embryonic lethal and correspondingly associated with ventral closure or early endodermal defects (Kuo et al., 1997; Molkentin et al., 1997; Morrisey et al., 1998; Koutsourakis et al., 1999). Thus, in at least some cases, both GATA4 and 6 have specific, crucial roles in development, and cannot compensate for each others' loss.

Partly because of the early lethality of the null mutants, the specific functions of GATA4 and 6 within the mammalian heart have been difficult to assess. Nonetheless GATA4 null embryos were shown to develop splanchnic mesoderm, which differentiated into cardiomyocytes expressing contractile proteins (Kuo et al., 1997; Molkentin et al., 1997). Similarly, GATA6 null ES cells differentiated into cardiomyocytes both in embryoid bodies in vitro, and in chimaeric mice, where they contributed to heart structures up to at least E10.5 (Koutsourakis et al., 1999). More recently, the early extraembryonic endoderm deficiencies of GATA4 and 6 null embryos have been rescued by tetraploid embryo complementation to support their gastrulation. The GATA4$^{-/-}$ rescued embryos arrested by E10, and displayed heart morphogenic defects including disrupted looping and septation, and a loss of the proepicardium (Watt et al., 2004). The rescued GATA6$^{-/-}$ embryos similarly survived only until E10.5 and were consistently smaller than control embryos, possibly due to impaired cardiovascular function, and the extent of ventricular trabeculation was in some cases reduced (Zhao et al., 2005). In both the GATA4$^{-/-}$ and GATA6$^{-/-}$ embryos, however, no overt disruption of cardiac GATA-dependent gene expression was observed. GATA5 null embryos, by contrast, were viable and their hearts developed normally (Molkentin et al., 2000). A requirement for a specific GATA-binding activity in the differentiation of the myocardium per se has therefore not been demonstrated in mammals by contrast to *Xenopus* and zebrafish (Reiter et al., 1999; Peterkin et al., 2003). Thus, there appear to be species differences in the requirement for a specific GATA-binding activity to effect cardiomyocyte differentiation.

While a large number of late cardiac-expressed structural genes have now been shown to be regulated by GATA-factors, little is known of the very earliest targets of GATA regulation in differentiating cardiomyoctes. Very early molecular events in mammalian cardiogenesis are currently difficult to elucidate using in vivo systems. Consequently, we (Brewer et al., 2005) and others (see (Anisimov et al., 2002) and references therein) have used the P19 embryonal carcinoma (EC) cell line as an in vitro mammalian model system for cardiomyocyte differentiation. Both serial analysis of gene expression (SAGE) (Anisimov et al., 2002) and microarray (Peng et al., 2002) analyses performed on P19 cells before and after differentiation have validated this system as a model of cardiogenesis. We have here utilised a clonal derivative, P19-CL6, which can be induced to differentiate highly efficiently (typically > 80%) along the cardiogenic pathway (Habara-Ohkubo, 1996). We have shown previously that overexpression of GATA4 or 6 early during the cardiac differentiation pathway, is sufficient to elicit premature expression of Nkx2.5, an early marker of precardiac mesoderm (Brewer et al., 2005). We show here

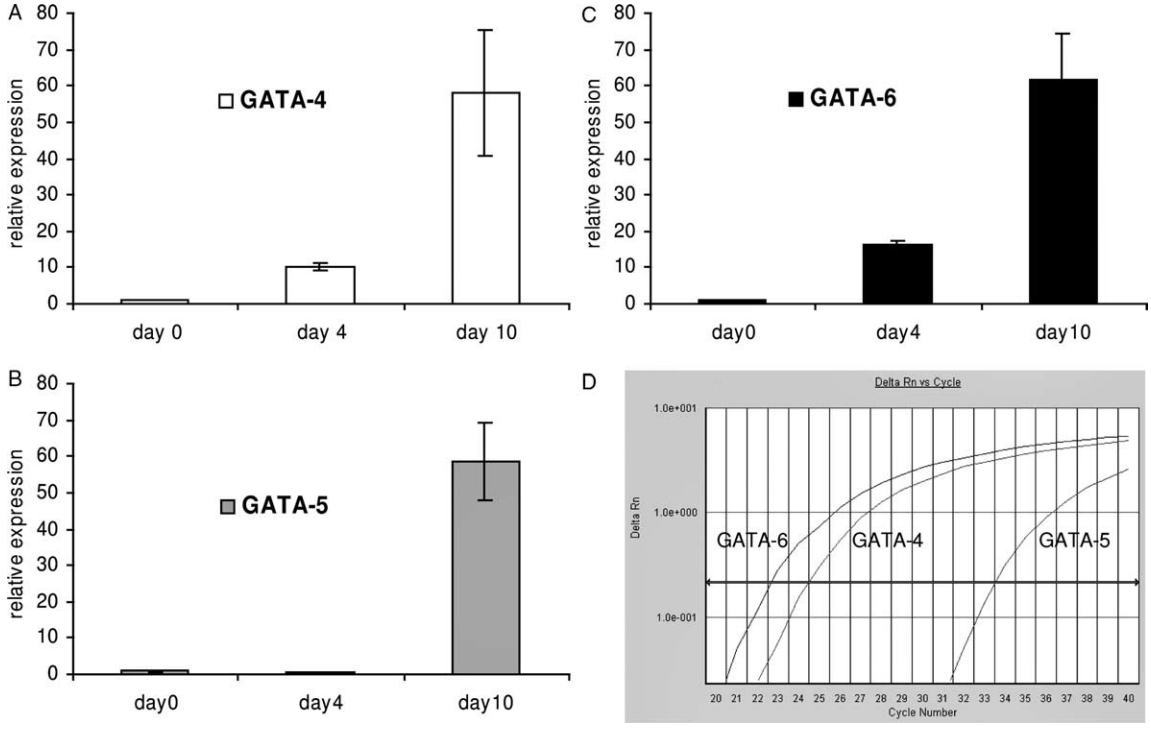

Fig. 1. Time course of expression of GATA factors during P19-CL6 cell differentiation. (A–C) qPCR analysis of mRNAs isolated from differentiating P19-CL6 cells at the time points indicated. The levels of expression are normalised to those in uninduced cells in each case. (D) qPCR run profiles of cDNA isolated from P19-CL6 cells at day 4 of induction using GATA-specific primers.