Keeping it simple: what mouse models of Wolf-Hirschhorn syndrome can tell us about large chromosomal deletions

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Wolf-Hirschhorn syndrome (WHS), caused by deletions on chromosome 4p16.3, is a contiguous gene syndrome, since it is caused by abnormalities in two or more genes that are adjacent to each other on a chromosome. Also, it is the first recognized subtelomeric deletion syndrome, because it results from a deleted portion of the chromosome next to the telomere cap. First described by Hirschhorn in 1961, WHS has an incidence of between one in 20,000 and one in 50,000 births. The major symptoms of WHS include mental retardation, epilepsy, growth delay, skeletal malformations and cranio-facial dysgenesis (Copper and Hirschhorn, 1961; Bergemann et al., 2005; Simon and Bergemann, 2008). Fine mapping of deletions from different WHS patients has led to the identification of two critical regions on chromosome 4p, Wolf-Hirschhorn syndrome critical regions 1 and 2 (WHSCR-1 and WHSCR-2) (Wright et al., 1997; Zollino et al., 2003). One or more genes located inside these WHSCRs are believed to be responsible for the pathogenesis of WHS. Since no single gene that has been identified is fully responsible for the complete spectrum of WHS phenotypes, it is widely accepted that WHS has a multigenic etiology. Adding to this complexity is emerging evidence that deletions of linked genes outside of the WHSCRs can contribute to both the onset and severity of the disease (Faravelli et al., 2007).

Different mouse models carrying targeted deletions of genes within WHSCRs have been established and they display phenotypes that are remarkably concordant with those of human patients. These mouse models show a significant likelihood of genetic interactions between certain candidate genes. However, each mouse model recapitulates only part of the constellation of phenotypes seen in human WHS and the specific genes contributing to each phenotype remains elusive (Simon and Bergemann, 2008).

The FGFRL1 gene encodes a member of the fibroblast growth factor (FGF) receptor family (Kim et al., 2001). The gene is located on 4p16.3, outside and distal to the WHSCRs. FGFRL1 was implicated recently as a candidate gene contributing to the craniofacial phenotype of a WHS patient (Engbers et al., 2009). A previous study found that targeted disruption of Fgfrl1 in the mouse resulted in diaphragm defects, but skeletal development was normal (Baertschi et al., 2007). In the previous issue of DMM, a study by Catela et al. reported a novel Fgfrl1 mouse model that was generated by targeting different exons from those described previously by Baertschi and colleagues (Catela et al., 2009). These Fgfrl1–/– mutant embryos display a broad array of phenotypes that are remarkably consistent with those of WHS patients. This disease model by Catela et al. thus provides a powerful tool to further dissect the complex etiology of WHS in the context of FGF signaling.

First, the authors examined the expression pattern of Fgfrl1 during mouse embryonic development. They reported strong expression at early time points in the brain, cranial placodes, pharyngeal arches, heart and somites (see fig. 1 in Catela et al.). Second, to explore the role of this gene in development, they generated a mouse knockout by homologous recombination in embryonic stem (ES) cells and verified their generation of a null allele (see fig. 2 in Catela et al.). Beginning at embryonic day (E)16.5, the authors began observing defects in their mutant embryos, which they classified as either mild or severe. Mild phenotypes included a dome-shaped cranium and short stature. These embryos survived until term, but died at birth owing to respiratory defects resulting from an abnormal diaphragm. Severely affected embryos, however, died at E18.5 because of severe vascular abnormalities and developmental retardation (see fig. 3 in Catela et al.).

Within the categories of mild and severe phenotypes, the authors further categorized the mutant embryos based on morphology. They focused on skeletal malformations, cardiovascular abnormalities and hematopoietic defects; all prominent features of human WHS. In mildly affected embryos at E18.5, Alcian Blue and Alizarin Red S staining revealed hypoplasia of all skeletal elements (see fig. 4 in Catela et al.). Specific defects in the axial and appendicular skeleton, along with deformities of the small vertebrae, pelvic girdle and rib cage, were comparable to the skeletal defects seen in WHS patients. Similar to the craniofacial defects in WHS, Catela et al. also observed hypoplasia and delayed fusion of the bones making up the cranial base of the skull in these embryos.

The authors noted defective blood distribution in the severely affected embryos and, thus, investigated the potential role of Fgfrl1 in cardiovascular development (see fig. 5 in Catela et al.). They observed a prominent ventricular septation defect arising between E12.5 and E14.5, which probably contributes to the prenatal lethality. The Fgfrl1–/– embryos also exhibited more subtle defects in cardiac valve structure. Together with Fgfrl1 expression in endocardial cushions (embryonic structures that give rise to mature heart valves), this finding suggests a role for Fgfrl1 in cardiac valve morphogenesis. Finally, the mutant embryos exhibited a placental defect, which may be secondary to the cardiac abnormalities (see fig. 6 in Catela et al.).

Examination of the hematopoietic system revealed decreased levels of red blood cells, hematocrit and hemoglobin in Fgfrl1-deficient embryos (see fig. 7 in Catela et al.). This is clinically relevant since the current detection of WHS relies on only gross observation of morphological defects. A method for evaluating children with abnormal blood cell counts could complement...
current practices to lead to more confident diagnosis of the disorder.

This WHS mouse model provides a powerful tool to study the mechanisms of human WHS. Implicating the FGF signaling pathway provides a good starting point with which to study WHS, but this is only the beginning. Further studies need to determine the molecular function of Fgfrl1, as well as its regulators, targets and potential interacting proteins. Crosstalk between signaling pathways, such as with the sonic hedgehog pathway, which has been shown to interact with FGF signaling in mouse models of DiGeorge syndrome (Brown et al., 2004), could be of interest in the WHS phenotype.

Since the Fgfrl1-null model described by Catela et al. does not recapitulate all aspects of the human phenotype, there may be additional modifier genes related to the syndrome that have yet to be discovered. Furthermore, the phenotypic variation between mice and humans might also be the result of the genetic differences between the organisms; thus, more genetic information from WHS patients is needed to confirm the human loci that contribute to the disease.

Although individually targeted gene deletions in the mouse, such as the one described here, are informative (Simon and Bergemann, 2008), larger scale chromosome deletions in the mouse might better mimic the human deletion syndrome. Indeed, a similar chromosome engineering approach was used to model DiGeorge syndrome, which led to the discovery that the TBX1 gene probably plays a crucial role in the disease (Lindsay et al., 1999; Lindsay et al., 2001; Merscher et al., 2001). Thus, the technology is now available for manipulating mouse chromosomes to mimic large deletion syndromes that occur in humans (van der Weyden and Bradley, 2006). Using a combination of engineered large chromosomal deletions and single gene knockouts in the mouse will be a powerful approach to model WHS and elucidate disease mechanisms. Although many interesting questions remain, this model gives researchers a great tool to study WHS, and it shows that the symptoms of related chromosomal deletion diseases might be largely because of only one, or a few, of the genes within the deletion.

REFERENCES