DiGeorge Syndrome, Tbx1, and Retinoic Acid Signaling
Come Full Circle

Katherine E. Yutzey

Among the most well-characterized cardiac congenital malformations are those associated with DiGeorge syndrome (DGS)/velocardiofacial syndrome. DGS is mainly caused by heterozygous deletion of a region of chromosome 22q11.2 and is characterized by cardiac conotruncal malformations, aortic arch anomalies, dysmorphic face and hypoplasia of the thymus and parathyroid.1 This spectrum of anomalies is attributed to defects in neural crest–derived structures and associated tissues. The T-box–containing transcription factor Tbx1 has been identified as responsible for cardiovascular, thymic, and parathyroid phenotypes of DGS.2 Alternations in retinoic acid (RA) metabolism are similarly associated with cardiac conotruncal, aortic arch, and pharyngeal structural abnormalities similar to DGS.3 In this issue of Circulation Research, Ryckebusch et al4 establish a new functional link between Tbx1 and RA signaling in the regulation of aortic arch anomalies in a mouse model of DGS.

A characteristic feature of DGS is malformation of the pharyngeal arch arteries (PAAs), particularly of the fourth PAA, which leads to interrupted aortic arch type B (IAA-B). Mice heterozygous for Tbx1 exhibit defects in the development of the fourth PAA related to aortic arch anomalies including IAA.5,6 Ryckebusch et al define molecular and cellular mechanisms underlying aortic arch anomalies in Tbx1+/− mice.14 The fourth PAA in embryonic day 10.5 embryos is derived from neural crest cells and is absent or hypomorphic in Tbx1+/− embryos. This PAA anomaly is accompanied by neural crest migration defects as well as inhibition of vascular smooth muscle (VSM) differentiation. Both neural crest migration and VSM differentiation related to PAA defects are controlled by a precise balance of RA signaling and Tbx1 expression. Studies reported here and elsewhere support a mutual inhibition of RA signaling and Tbx1 expression (Figure). Reduced RA signaling with heterozygosity of retinaldehyde dehydrogenase 2 (raldh2) improves fourth PAA development in Tbx1+/− embryos through restoration of normal neural crest migration trajectories and increased VSM differentiation. The Tbx1-RAR interaction does not appear to affect second heart field derivatives but specifically contributes to neural crest-related anomalies. A significant conclusion from this work is that decreased RA signaling in a mouse model of DGS ameliorates aortic arch anomalies. Together, these studies provide important mechanistic insights into the molecular regulatory interactions that lead to common cardiac malformations in the human population.

Extensive efforts by multiple research groups led to the identification of Tbx1 as a disease causing gene in DGS.2 Mice lacking Tbx1 have severe PAA anomalies, as well as cardiac conotruncal, pharyngeal, and facial defects related to DGS.5,7,8 However, Tbx1+/− heterozygous mice exhibit less severe PAA defects than those observed with the 22q11.2 deletion.9 Therefore, it is hypothesized that modifier genes contribute to DGS phenotypes associated with Tbx1 heterozygosity. This idea is supported by the variable spectrum of malformations and penetrance of DGS, even in the same family. There is increasing evidence that RA signaling is a critical modifying factor in DGS. Vitamin A (retinoic acid) deficiency has long been associated with cardiovascular malformations in humans and rodents.9,10 Increased or decreased RA signaling in mice leads to DGS phenotypes evident in pharyngeal arch anomalies, including IAA, and cardiac conotruncal defects.3 Mice with decreased expression of raldh2, a rate-limiting enzyme in RA synthesis, exhibit DGS-related PAA defects.1 Mice with loss of Tbx1 expression leads to similar pharyngeal, craniofacial, and cardiovascular anomalies related to abnormal development of neural crest or second heart field derivatives.

Evidence from multiple systems supports a mutually repressive regulatory interaction between RA signaling and Tbx1 in the control of neural crest migration and differentiation in the pharyngeal arches and cardiac outflow tract (OFT) (Figure). The study by Ryckebusch et al shows that Tbx1 expression is increased in raldh2−/− embryos or in pharyngeal arch tissue treated with the RA inhibitor disulfiram. Similarly, Tbx1 expression is repressed by RA treatments. Previous studies in chicken embryos also showed that RA treatment represses Tbx1 expression in the developing pharyngeal arches.11 Mice lacking Tbx1 have increased expression of raldh2, as well as reduced expression of RA inactivation enzymes cyp26a1, -b1, and -c1, with an overall increase in RA signaling evident in ectopic activation of RARE reporter gene.12,13 Overall, these data demonstrate that Tbx1 represses RA signaling, whereas increased RA signaling leads to decreased Tbx1 function. Therefore, a precise balance of Tbx1 function and RA signaling is necessary for normal pharyngeal and cardiac conotruncal development.
Additional modulators of Tbx1 function also contribute to DGS phenotypes (Table). The CRK-like (CRKL) gene, which encodes an adaptor protein implicated in tyrosine kinase signaling, also is located on chromosome 22q11.2.13 Mice lacking Crkl have PAA and cardiac OFT defects related to DGS, but Crkl−/− mice are apparently normal.14 A genetic interaction for Tbx1 and Crkl is supported by the observation that Tbx1+/−;Crkl−/− mice have more severe aortic arch, thymic, and parathyroid defects than Tbx1+/− mice.14 In addition, RA signaling is increased in Crkl−/− mice, and reduced RA signaling in Tbx1−/−;Crkl−/−;Raldh2−/− mice decreases the penetrance of thymic hypoplasia in these animals.13 Decreased fibroblast growth factor (FGF)8 function also enhances DGS phenotypes in Tbx1−/− mice, as well as Tbx1+/−;Crkl−/− mice.13,15 The direct and indirect regulatory relationships of Tbx1, Crkl, FGF8, and RA signaling in the regulation of neural crest derivatives in the pharynx and cardiac OFT have not yet been fully established (Figure). Tbx1 and Fgf8 are expressed together in the pharyngeal pouches, and Tbx1 expression also is predominant in the pharyngeal mesenchyme.15 Crkl is expressed in the migrating neural crest cells, and loss of Crkl disrupts Fgf8 signaling in neural crest derivatives.14,16 It is likely that these factors act by cell-autonomous and nonautonomous mechanisms to regulate neural crest migration and differentiation in the developing pharynx and cardiac OFT.

Recent progress has been made in defining the genetic, molecular, and cellular interactions that contribute to DGS-related cardiovascular and pharyngeal phenotypes. These studies have identified genetic lesions in mice that act as positive or negative modifiers of clinically significant DGS-related congenital malformations. This information could be exploited therapeutically in the genetic diagnosis of DGS and associated lesions. Once specific modifier mutations have been identified in the human population, it may be possible to define combinations of gene mutations that are predictive of specific malformations. Currently, the genetic basis for the high variability of DGS phenotypes and reduced penetrance seen in patients is not known.

Multiple signaling pathways, including RA and FGF8, contribute to DGS-related congenital malformations and could possibly be exploited in the treatment of the syndrome. However, there are several obstacles to translation of this knowledge into the clinic. The developmental origins of DGS-related cardiac defects occur during the first trimester of pregnancy, and by the time the lesions can be detected, they are no longer amenable to treatment. The study by Ryckebusch et al establishes a complex link between Tbx1 and RA signaling in development of PAA anomalies associated with DGS.4 It is apparent that the levels, timing, and localization of RA signaling are critical for normal development of the PAA and cardiac OFT. RA signaling can manipulated pharmacologically, but these drugs are known to cause multiple congenital malformations in many developing organ systems and are prohibited from use during pregnancy.17 Additional signaling pathways implicated in development of pharyngeal arches and cardiac OFT also are critical at different times and in different organ systems during development. Therefore the challenge for the future is the translation of our recent in depth molecular knowledge to clinical applications related to congenital heart disease with roots in early embryonic development.

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**Disclosures**

None.

**References**


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Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>DGS</td>
<td>DiGeorge syndrome</td>
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<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
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<tr>
<td>IAA</td>
<td>interrupted aortic arch</td>
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<tr>
<td>OFT</td>
<td>outflow tract</td>
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<tr>
<td>PAA</td>
<td>pharyngeal arch artery</td>
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<tr>
<td>RA</td>
<td>retinoic acid</td>
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<td>SHF</td>
<td>second heart field</td>
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<td>VSM</td>
<td>vascular smooth muscle</td>
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**Table. Mouse Mutants With Cardiac and Pharyngeal Malformations Related to Human DGS**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Malformation</th>
</tr>
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<tbody>
<tr>
<td>Tbx1+/−</td>
<td>Fourth aortic arch anomaly (reduced penetrance)</td>
</tr>
<tr>
<td>Tbx1−/−</td>
<td>Aortic arch anomalies, cardiac outflow tract defects, ventricular septal defect, hypoplastic thymus and parathyroid glands, abnormal facies, cleft palate</td>
</tr>
<tr>
<td>raldh2 hypomorph</td>
<td>Aortic arch anomalies</td>
</tr>
<tr>
<td>Tbx1+/−;raldh2+/−</td>
<td>Regression of fourth aortic arch anomaly</td>
</tr>
<tr>
<td>Crkl+/−</td>
<td>No apparent anomalies</td>
</tr>
<tr>
<td>Tbx1+/−;Crkl+/−</td>
<td>Hypoplastic thymus, aortic arch anomalies, parathyroid defects</td>
</tr>
<tr>
<td>Tbx1+/−;Crkl+/−;raldh2+/−</td>
<td>Restoration of hypoplastic thymus</td>
</tr>
<tr>
<td>Tbx1+/−;Fgf8+/−</td>
<td>Aortic arch anomalies (high penetrance)</td>
</tr>
<tr>
<td>Crkl+/−;Fgf8+/−</td>
<td>Hypoplastic thymus, parathyroid defects</td>
</tr>
</tbody>
</table>

See the text for references and details.


**Key Words:** DiGeorge syndrome ■ Tbx1 ■ retinoic acid ■ aortic arch malformations
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