Modeling Development of the Epicardium and Coronary Vasculature

In Vitro Veritas?

Cathy J. Hatcher, Craig T. Basson

“Your vision will become clear only when you can look into your own heart... Who looks outside, dreams; who looks inside, awakens.”—Carl Jung

The ontogeny and interrelationships of the epicardium surrounding the heart and the vascular beds and conduction pathways investing it remain fundamental mysteries of cardiovascular development. Although several molecules and discrete signaling events have been identified, we lack a detailed schematic of the relevant molecular genetic cascades. Genetic etiologies for congenital heart defects affecting the epicardium and the coronary vasculature have yet to be defined. Successful clinical manipulation of stem cells from a variety of sources in order to recreate blood vessels and conduction tissues in the diseased adult heart would be greatly enhanced by the discovery of a Rosetta stone to decode the key biochemical events and their interactions that comprise such embryonic cardiovascular processes.

In order to define molecular regulation of coronary vasculogenesis and Purkinje fiber development in the heart, investigators have largely relied on chick and mouse models of cardiogenesis. These animal models have led them to look outside the primitive heart tube for the earliest events that initiate the establishment of these tissues. Such studies have highlighted the contribution of the proepicardial organ (PEO). The PEO (Figure) is an outgrowth of a cluster of extracardiac epithelial cells on the dorsal body wall adjacent to the heart tube’s atroventricular canal. Cells from the PEO are the precursors for several lineages within the heart including epicardial epithelial cells, myocardial connective tissue cells, coronary vascular smooth muscle cells, and coronary endothelial cells. Moreover, communication between the progeny of PEO cells and cardiomyocytes provides instructions for the differentiation of Purkinje fibers.

Several morphological activities are involved in the embryonic evolution of PEO cells. Cells within the PEO proliferate and then migrate out of the organ to travel over the myocardial surface of the looping heart tube. Villous projections of PEO cells extend over the posterior part of the ventricles and the atrioventricular sulcus. These migrating PEO cells differentiate into a static epithelium that comprises the primitive epicardium. The epicardial layer covers the embryonic myocardium up to the border between the myocardial and mesenchymal regions of the outflow tract. Epicardial cells synthesize a dense layer of extracellular matrix that resides between them and the myocardium. Ultimately, a subpopulation of epithelial cells migrate out of the epicardium into the subepicardial space, and these migrating epicardial cells undergo an epithelial-to-mesenchymal transformation (EMT). The resultant mesenchymal cells invade the actively proliferating myocardium and give rise to cardiac fibroblasts and coronary vasculature. A subgroup of these cells interact further with the myocardial cells and influence myocyte differentiation into Purkinje cells.

EMT is a critical developmental switch that occurs in several organs. The transcription factor WT-1 contributes to this process, and knockout of WT-1 in the mouse leads to deficiencies in EMT and congenital defects in the urogenital system, adrenal gland, and heart. The hearts of WT-1 knockout mice lack epicardium and coronary vessels. Vascular cell adhesion molecule (VCAM-1) and α4 integrin knockout mice exhibit similar cardiac defects. Several signaling mechanisms have been shown to modify EMT in the heart. For instance, Morabito et al demonstrated in chick cells that factors affecting the epicardium and EPDC development. In contrast to rat and mouse embryos, in which the PEO exists as a diffuse extension of the septum transversum, the chick and quail PEO exists as a free structure able to be manipulated in ovo or easily microdissected and isolated for in vitro studies without fear of potential contamination by non-PEO cells. Yet, establishment
of efficient experimental systems for the study of the mammalian PEO and EPDCs remained an important, unattained goal. In an effort to devise a model of mammalian EPDC differentiation, Eid et al.\textsuperscript{10} isolated epicardial mesothelial cells (EMCs) from the epicardium of adult male rats. Their work was instrumental in providing one of the first descriptions of cellular interactions between epicardial cells and ventricular myocytes in culture, and it provided researchers with a potentially unique opportunity to analyze epicardial differentiation and vasculogenesis in vitro. In their initial description of the EMC line, Eid et al.\textsuperscript{10} demonstrated that physical interactions between EMCs and adult rat ventricular myocytes aided myocyte differentiation and stimulation of high-amplitude synchronous contractions in vitro by the establishment of intercellular gap junctions. They proposed that such interactions might have physiological relevance during embryogenesis before coronary vasculogenesis, a stage when the myocardial myoblasts are in close contact with epicardial cells. Although the EMC line offered all the technical advantages of an established cell line that facilitates experimentation to confirm this hypothesis, the ability of this transformed and selected population of cells to mimic physiological processes remained an unanswered question.

In this issue of *Circulation Research*, Wada and colleagues\textsuperscript{11} demonstrate that EMCs, in fact, can mimic at least some components of epicardial differentiation and coronary vasculogenesis. They show that EMCs retain their ability to produce mesenchyme in response to specific growth factors and are able to generate smooth muscle cells. EMCs form an organized epithelium in culture and express proteins (E-cadherin, β-catenin) associated with adherens junctions as well as proteins (ZO-1) associated with tight junctions. Bves, an epicardial cell marker described by the same laboratory, is also expressed by EMCs and colocalizes with E-cadherin. Several genes (WT-1, Tbx18, connexin-43, and cytokeratins) whose expression patterns are enriched in epicardium—albeit expressed in other tissues as well—are expressed by EMCs. In the presence of serum-containing medium supplemented with EGF or bFGF, individual cells developed an elongated fibroblast-like contour and lost contact with the epithelial monolayer. Simultaneous disruption of adherens junctions indicated by redistribution of E-cadherin was also observed. Wada et al.\textsuperscript{11} propose that these events represent an in vitro correlate of EMT in vivo. Importantly, expression of α-smooth muscle actin, calponin, and α-smooth muscle tropomyosin mRNA in a subpopulation of mesenchyme-like cells suggested that at least some EMCs retained the capacity to differentiate along a smooth muscle lineage. Thus, Wada et al.\textsuperscript{11} propose that EMCs may provide an easily manipulated in vitro model of EPDC differentiation and coronary vasculogenesis.

These investigators have set the stage for further mechanistic analyses; their work shows that the EMC line can be an invaluable tool for investigators interested in cardiac and vascular development. Certainly, biochemical signaling events triggered in the EMT of EMCs will be elucidated in future studies. The task ahead, though, will include delineating the limitations of the EMC line in modeling events both upstream and downstream from epicardial development.

What characteristics of proepicardial cells are retained by this cell line that already exhibits features of an organized epithelium? Can studies of EMCs shed light on PEO development, expansion, and dispersion that are required for formation of the epicardial layer? As we consider events in organogenesis that follow creation of the epicardial layer, the detailed characterization of the EMC line by Wada et al.\textsuperscript{11} hints at other unresolved issues. Less than 5% of EMCs retain the ability to differentiate along a smooth muscle lineage, and functional competence of these cells needs to be demonstrated. To date, conditions have not been defined that drive EMC differentiation into endothelial cells, and it remains unknown whether the cell line retains this capacity. Investigators will need to characterize both molecular and physiological markers to distinguish among EMC subpopulations that may be analogous to specialization of EPDCs in vivo. Definition of such profiles will foster efforts to utilize EMCs to unravel biochemical and genetic cascades that trigger epithelial commitment, vascular cell differentiation, and Purkinje fiber induction. Furthermore, dynamic models of EMC function and movement may be devised that will shed light on epicardial cell motility that, in vivo, places EPDCs in apposition with myocardial cells and sets the stage for transformations required for coronary vascular and Purkinje fiber development.

Future investigation of EMCs and other EPDC models may also expand our definition of this cell population. To date,
studies have considered EPDCs and cardiomyocytes as distinct lineages whose interactions have important developmental consequences. However, evolving data from van den Hoff and colleagues suggest that, at least in vitro, epicardial cells may also be able to differentiate along a cardiomyocyte lineage as well as the vascular path considered by Wada et al. Whether such differentiation occurs in vivo during cardiogenesis is unknown. Taken together, though, current research highlights the extraordinary plasticity of proepicardial and epicardial cells. As we map out the intersecting molecular genetic pathways that regulate their differentiation, we may ultimately find these populations a rich source of progenitor cells to be clinically manipulated in order to regenerate a wide array of impaired structures and functions in the ailing heart.

References

12. Kruthof BPT, Somi S, Moorman AFM, van den Hoff MJB. Myocardium Formation After the Initial Development of the Linear Heart Tube (Proepicardial Cells Have the Potential to Differentiate Into Cardiomyocytes) [dissertation]. Amsterdam, the Netherlands: University of Amsterdam, Department of Anatomy & Embryology, Academic Medical Center; 2003.

KEY WORDS: epicardium ■ coronary arteries ■ tissue culture ■ heart development
Modeling Development of the Epicardium and Coronary Vasculature: In Vitro Veritas?
Cathy J. Hatcher and Craig T. Basson

*Circ Res.* 2003;92:477-479
doi: 10.1161/01.RES.0000064380.47325.D4
*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/92/5/477

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org//subscriptions/