血管は、酸素や栄養を運搬するという重要な機能を果たしながら、胚のかたち作りにあわせて絶えずリモデリングを繰り返し、最終的な成体型の血管ネットワークへと近づいていくことが知られている。しかし、それらの過程がどのような内皮細胞のダイナミックな動きによって支えられているのかは謎である。血管網が形成されるしくみを個々の細胞の挙動の観点から直接的に理解する為、血管内皮細胞を特異的に蛻光タンパク質で標識するトランスジェニックウズラ鶏を用いて、イメージング解析を行っている。血管形成を支える個々の細胞の動きを明らかにするとともに、それらの動きを制御する分子メカニズムを解明する。

Blood vessels play an important role to deliver oxygen and nutrition in the body. Their network pattern is formed by the organization of the endothelial cells provided by mesodermal tissues during embryonic development. Although a number of molecules required for blood vessel formation have been identified mainly by gene inactivation studies in mice, a detailed understanding of normal blood vessel formation and the direct effects of the genetic abnormalities have not been well performed.

To take advantage of the easy accessibility to the developing embryo, Japanese quail is exploited as a model system to study blood vessel formation of higher vertebrates by a dynamic imaging approach. A transgenic quail carrying nuclear-localizing fluorescent protein with endothelial-specific promoter enables vital imaging of the endothelial cells in vivo. Using this model animal, we are studying molecular mechanisms of dynamic cell behaviors during blood vessel formation.


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Androgen Regulates Dimorphic F-Actin Assemblies in the Genital Organogenesis

Liping Liu, Kentaro Suzuki, Eunice Chun, Aki Murashima, Yuki Sato, Naomi Nakagata, Toshihiko Fujimoto, Shigenobu Yonemura, Wanzhong He, Gen Yamada

*National Institute of Biomedical Engineering, Chinese Academy of Sciences and **National Institute of Biomedical Sciences, Beijing, China; †Department of Developmental Genetics, Yokohama Medical University, Yokohama, Japan; ‡Institute of Human Embryology, Department of Anatomy, Iwate Medical University, Nishinokuka-ya-hata, Department of Anatomy and Cell Biology, Kyoto University Graduate School of Medicine, Fukuoka, Japan; §Division of Reproductive Engineering, Center for Assisted Reproduction and Development, Kumamoto University, Kumamoto, Japan; ¶Division of Embryology, National Institute for Basic Biology, Okazaki, and ‖Department of Cell Biology, Tokushima University Graduate School of Medical Science, Tokushima, Japan

Abstract

Androgen activity induces a distinctive sexual differentiation of the male reproductive tract, including hypospadias, an abnormal formation of the penile urethra. Androgen signaling in the urethral mesenchyme cells (UMCs) plays essential roles in driving masculinization of the urethra. However, cellular events for sexual differentiation remain virtually unknown. In this study, histological analyses, fluorescence staining, and transmission electron microscopy (TEM) were performed to reveal the cellular dimorphisms of UMCs. F-actin dynamics and migratory behaviors of UMCs were further analyzed by time-lapse imaging. We observed a prominent accumulation of F-actin with poorly assembled extracellular matrix (ECM) in female UMCs. In contrast, thin filaments of F-actin co-occurring with the ECM through membrane receptors were identified in male UMCs. Processes for dimorphic F-actin assemblies were temporally identified during androgen-regulated masculinization programming window and spatially distributed in several embryonic reproductive tissues. Stage-dependent modulation of the F-actin sexual patterns by androgen signaling is also demonstrated by time-lapse analysis. Moreover, androgen regulates coordinated migration of UMCs. These results suggest that androgen signalng regulates the assembly of F-actin from cytoskeletal arrangements of individual filaments. Such alteration appears to promote the ECM assembly and the mobility of UMCs, contributing to male type genital organogenesis.

KEYWORDS: Fibronectin, Filopodia, Somite, Enderdem, Dorsal aorta

INTRODUCTION

Three-dimensional (3D) organization of the extracellular matrix (ECM) is crucial for configuring tissue and organ shape and function (Raynor and Raynor, 2010). Understanding how the ECM is patterned and how cells sense and react to ECM patterning are key to reconstituting the cellular and molecular mechanisms driving tissue morphogenesis. Several studies have shown that physical properties of the ECM, including geometry, rigidity and tension, may influence the forces exerted on cells, which in turn affect cell shape and ultimately lead to the modification of fundamental cellular processes such as migration and differentiation in vivo (Dufort et al., 2011). However, due to dynamic morphogenesis during embryo development, the interplay between cell cytoskeletal architecture and ECM properties and related tensional forces is poorly understood.

Fibronectin (FN) is an ECM component that is essential for the morphogenesis of various tissues and organs (Rostovtsev and Delisniece, 2010). The generation of FN filaments is dependent on the activity of actin filaments and the ability to recruit FN to the actin cytoskeleton. In addition, intrinsic and extrinsic mechanical forces can promote FN fibrillarization and fibronectin alignment along the direction of force (Bavesey et al., 2001). Fawzy et al., 2008; Hutton and Winfield, Fawzy, 2016). Given that FN filaments can stretch 5-6 fold in vivo (Lettie et al., 2008; Brown et al., 2007), it is imperative to speculate that they could be involved in matrix elasticity between separated tissues.

In early somite segments, the somatic mesoderm and endoderm are separated by a 40–50 nm gap. The paired dorsal somite andes move through this gap (Sato, 2015). Here, we report that the gap between somites and endoderm is spanned by fillopod-like extensions of FN filaments, together with long fillopods extending from the dorsal surfaces of somite epithelial cells. We discovered three FN filaments by studying FN distribution in a transgenic qld embryo model system, using tissue-specific DNA demethylation and time-lapse imaging (Bower et al., 2010). The P0QRBQMBMcCherry transgenic qld embryo enables us to mark every cell and ECM dynamics over time in living embryos, and to study cell-autonomous roles of extracellular genes (Huss et al., 2011). This approach allowed us to visualize the previously unknown pattern of FN in the somites-endoderm gap. In striking contrast to previously reported patterns of FN distribution near the basal surfaces of cells, we found that the FN filaments extended far away from cell bodies into the gap space. The directional arrangement of the FN filaments and their accompanying somite cell (fillopodia) suggested that they might be patterned by a site-specific tension force. Subsequent experiments suggest that cytoskeletal forces associated with the incorporation of the dorsal aortas are involved in formation of the FN filaments.

RESULTS

FN forms filaments between somites and endoderm. Using cellular and tissue-specific demethylated transgenic qld embryos as early stages and stage embryos, we observed that long FN filaments patterned as fillopodia (~50–60 μm in height) in the gap between somites and endoderm (lower panels in Fig. 1BC). FN around the somite epithelium is known to be required for somite boundary formation and cell rearrangement during somite epithelialization in various vertebrate embryos (Sato et al., 2011; Jolli et al., 2015; Koshida et al., 2009; Mann et al., 2009; Rhin
Sall1 Maintains Nephron Progenitors and Nascent Nephrons by Acting as Both an Activator and a Repressor

Shoichiro Kanda,* Shunsuke Tanigawa,* Tomoko Ohnori,* Atsuhito Taguchi,* Kaniko Kudo,* Yutaka Suzuki,* Yuki Satō,* Shigeki Hino,* Makiko Sander,* Alan O. Perantoni,* Sumio Sugano,* Mitsuaki Nakao,*** and Ryuchi Nishikakeru***

Departments of *Kidney Development and Medical Cell Biology, Institute of Molecular Embryology and Genetics, and **Priority Organization for Innovation and Excellence, Kumamoto University, Kumamoto, Japan; †Department of Medical Geriatric Sciences, University of Tokyo, Tokyo, Japan; Departments of Pediatrics and Cellular and Molecular Medicine, University of California at San Diego, La Jolla, California; Cancer and Developmental Biology Laboratory, National Cancer Institute, National Institutes of Health, Frederick, Maryland; and ***CREST, Japan Science and Technology Agency, Saitama, Japan

ABSTRACT

The balanced self-renewal and differentiation of nephron progenitors are critical for kidney development and controlled, in part, by the transcription factor Six2, which antagonizes canonical Wnt signaling-mediated differentiation. A nuclear factor, Sall1, is expressed in Six2-positive progenitors as well as differentiating nascent nephrons, and it is essential for kidney formation. However, the molecular functions and targets of Sall1, especially the functions and targets in the nephron progenitors, remain unknown. Here, we report that Sall1 deletion in Six2-positive nephron progenitors results in severe progenitor depletion and apoptosis of the differentiating nephrons in mice. Analysis of mice with a inducible Sall1 deletion revealed that Sall1 activates genes expressed in progenitors while repressing genes expressed in differentiating nephrons. Sall1 and Six2 co-occupied many progenitor-related genes in situ, and Sall1 bound to Six2 biochemically. In contrast, Sall1 did not bind to the Wnt4 locus suppressed by Six2. Six2-mediated repression was also independent of its binding to DNA. Thus, Sall1 maintains nephron progenitors and their derivatives by a unique mechanism, which partly overlaps but is distinct from that of Six2.

Six2 activates progenitor-related genes in Six2-positive nephron progenitors and represses gene expression in Six2-negative differentiating nascent nephrons.


Review Article

Transgenic Imaging in Birds, and available transgenic reporter lines

Yuki Sato1* and Rusty Lansford2*

1Priority Organization for Innovation and Excellence, Kumamoto University, 2-1-1 Hondo, Kumamoto, 860-0811, Japan; and 2Department of Radiology, Children's Hospital Los Angeles and the Keck School of Medicine at the University of Southern California, California, USA

Avian embryos are important model organisms to study higher vertebrate development. Rfly accessibility to developing avian embryos enables a variety of experimental applications to understand specific functions of molecules, tissues-tissue interactions, and cell lineages. The whole-mount or two-color technique for avian embryos permits time-lapse imaging analysis for a better understanding of cell behaviors underlying tissue morphogenesis in physiological conditions. To study mechanisms of blood vessel formation and remodeling in developing embryos using a time-lapse imaging approach, a transgenic quail model, Tg(Hbta1a::EYFP), was generated. From a cell behavior perspective, Tg(Hbta1a::EYFP) quail embryos are a suitable model to shed light on how the structure and pattern of blood vessels are established in higher vertebrates. In this manuscript, we give an overview on the biological and technological background of the transgenic quail model and describe procedures for the ex ovo culture of quail embryos and time-lapse imaging analysis.

Key words: blood vessels, confocal laser microscopy, quail, time-lapse imaging, transgenic bird.

Introduction

Time-lapse microscopy is an important approach to study dynamic morphogenetic events during embryogenesis on the cellular level. Confocal laser microscopy enables the capture of high-resolution images of cells in three-dimensions at regular intervals, permitting us to trace and quantify behaviors of the cells by means of computer image analysis (or a real-time working protocol can be done by hand). Quantitative analysis of cell behavior holds us to determine how a cell's individual behavior influences tissue shapes (i.e., when a gene function is disrupted). Time-lapse imaging and analysis of cultured cells are easy to perform because the cells can be labeled efficiently with fluorescent proteins by DNA transfection. In addition, because they are not multi-layered, the number of photomicrographs is minimized and light scattering is negligible. In contrast, time-lapse imaging of developing avian embryos is not achieved easily because large body sizes require several xy and numerous z-sections and the opaque tissue causes light scattering. In addition, drifting or "fuming" off of embryos frequently occurs since they are growing in all dimensions. These issues notwithstanding, capturing cell behavior in vivo is vital and important in the field of developmental biology because the tissue shape changes and functions are dynamically governed by physiological circumstances in a body. Time-lapse Imaging analysis of developing embryos requires an animal model that enables: (i) fluorescent labeling of desired cells; (ii) undergoes normal development on a microscopic stage and is resistant to the deleterious effects of excitation light; and (iii) amenable to experimental manipulations of its molecular and cellular components. Developing chick and quail embryos are easily observed by creating a small hole in the eggshell: extended observation is possible if the hole is transparently repaired using a cover slip, saran wrap, Dupont stuff. Researchers have taken advantage of this built-in culture system from hundreds of years ago (see Brien 2004 for a review), using avian embryos as a model for developmental biology. Time-lapse observation of migrating neural crest cells was performed for the first time in chick embryos in ovo by using fluorescent dye, for example, Rhodamine dextran.
Review Article

Dorsal aorta formation: Separate origins, lateral-to-medial migration, and remodeling

Yuki Sato*
Priority Organization for Innovation and Excellence, Kumamoto University, 2-2-1 Honjo, Kumamoto, 860-8511, Japan

Blood vessel formation is a highly dynamic tissue-remodeling event that can be observed from early development in vertebrate embryos. Dorsal aortas, the first functional intra-embryonic blood vessels, arise as two separate bilateral vessels in the trunk and undergo lateral-to-medial translocation, eventually fusing into a single large vessel at the midline. After this dynamic remodeling, the dorsal aorta generates hematopoietic stem cells. The dorsal aorta is a good model to use to increase our understanding of the mechanisms controlling the establishment and remodeling of larger blood vessels in vivo. Because of the easy accessibility to the developing circulatory system, quail and chick embryos have been widely used for studies on blood vessel formation. In particular, the mapping of endothelial cell origins has been performed using quail-chick chimeras analysis, revealing endothelial, vascular smooth muscle, and hematopoietic cell progenitors of the dorsal aorta. The avian embryo model also allows conditional gene activation/inactivation and direct observation of cell behaviors during dorsal aorta formation. This allows a better understanding of the molecular mechanisms underlying specific morphogenetic events during dynamic dorsal aorta formation from a cell behavior perspective.

Key words: dorsal aorta, endoderm, notochord, quail-chick chimera, somite.

Introduction

The developing chick embryo is easily observed by creating a small hole in the eggshell; extended observation is even possible if the hole is sealed by adhesive tape. Researchers have taken advantage of this built-in culture system for more than a century, using chick embryos as a model for developmental biology. The blood vascular network, which can be obtained in the chick embryo from day 2 of incubation, is one of the prominent features of vigorous embryogenesis. Moreover, a beating heart and flowing red blood cells are the first visible vital functions of the circulatory system; therefore, many biologists have been drawn to study the blood vessels in the chick embryo (for a review, see Noden 1990). Dorsal aortae are the first intra-embryonic blood vessels to arise in the trunk. Primary dorsal aortae comprise a pair of longitudinal vessels in which the anterior ends are connected to the nascent heart via outflow tracts and the posterior parts are linked to the main aorta at the umbilicus level. In addition to its critical function as the largest vessel to circulate blood through the embryo's body, the dorsal aorta is known to be a site of secondary embryonic hematopoiesis (for reviews, see Determan-Lieves & Le Douarin 2004; Jaffe et al. 2005; Adero & Garcia-Castroana 2012). The dorsal aorta also acts as a signaling center, providing instructive signals to induce pancreas differentiation, migration of neural crest cells, and subsequent specification of cell types (Peles et al. 1998; Schmid et al. 1999; Lammers et al. 2001; Yoshimoto & Zaret 2004; Saito et al. 2012). Moreover, the dorsal aorta does not resemble peripheral blood vessels, which are typically seen in small organs and cancer, in terms of its diameter, number of endothelial cells incorporated within the vessel, and its blood flow volume. Studies of dorsal aorta formation during embryogenesis will provide clues to understanding the mechanisms through which large blood vessel formation is achieved in vivo. This review presents the dynamic process of dorsal aorta formation and describes recent discoveries related to regulatory mechanisms that have been revealed by studies using avian (mostly quail and chick) embryo models.

*Author to whom all correspondence should be addressed. Email: sato@kumamoto-u.ac.jp
Received 2 September 2012; revised 19 September 2012; accepted 18 September 2012
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