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A recently developed technique enables quantitative study of the initiation of left-right asymmetry using cells grown on micropatterns with close appositional boundaries. It was found that mammalian cells exhibit either a left or right bias in their migratory behavior, which was determined by cell phenotype, different for certain cancer and normal cells, and dependent on functionality of the actin cytoskeleton. We discuss here the relevance of this simple technique to study of development and birth defects in laterality.

Morphogenesis is a biological process of arranging cells to form tissue with distinct architectures. Life often takes a form with certain symmetry, such as spherical (e.g., marine plankton), radial (e.g., sea anemone), or bilateral (e.g., human). Importantly, the symmetric body plan is often broken by chiral structure or asymmetric development and placement of paired organs, such as the anti-clockwise twining of climbing trees, the chiral helices of snail shell, and the rightward looping of human heart tube. Almost all human visceral organs display left-right (LR) asymmetry of their position and shape. The complete mirror-image reversal of internal organs (situs inversus totalis) is surprisingly rare.1 Birth defects in laterality often result from heritable genetic diseases such as Kartagener syndrome or prenatal exposure to teratogens.2 In some instances, birth defects are associated with disease such as breast cancer3 or maternal diabetes.4,5 Therefore, it is not only of scientific interest but also of great clinic importance to investigate the mechanisms associated with the establishment of LR asymmetry during development.

Using embryos from different animals, several models have been developed for establishing the LR asymmetry, such as voltage gradients resulting from asymmetric expression of ion channels (Fig. 1A),6,8,9 directional nodal flow driven by primary cilia (Fig. 1B),7,10 and asymmetric vesicular transport via unconventional myosin 1D along actin cable networks.11-13 However, the mechanism of the early LR symmetry breaking during development remains controversial. For instance, does the initiation of LR asymmetry rely on certain embryonic structures such as node? Is the LR asymmetry a fundamental property of the cell? How can intrinsic cell chirality, if exists, be translated to a multicellular asymmetric structure? To address these questions, it is of interest to examine whether a homogeneous cell population can express chirality in vitro.

Micropatterning chiral morphogenesis
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Left-Right Asymmetry in Micropatterned Cells
LR asymmetry of cell populations cultured on geometric patterns was recently demonstrated by our group4 in a way that provides direct evidence for intrinsic cellular chirality and relates this important cell property to chiral morphogenesis at a multicellular level. Over 10 different types of human and rodent cells were tested and shown to exhibit definite chirality when cultured on micropatterned rings and strips, independent of patterning surface chemistry and protein coating.
Interestingly, chirality depended on cell phenotype, and the cellular alignment resembled the chiral structures found in nature such as dextral and sinister snail shells (Figs. 2A-B). For instance, the C2C12 cells express counter-clockwise (CCW) alignment, which is defined for the cells pointing outwards when followed in a CCW direction (Fig. 2C). The human umbilical vein endothelial cells (hUVECs), in contrast, exhibit a clockwise (CW) alignment (Fig. 2D).

The observed chiral alignment is closely associated with cell polarization and directional migration at boundaries. Micropatterned cells polarize at boundaries by positioning their centrosomes and Golgi apparatus, rather than their nuclei, toward each boundary (Figs. 2C–E). This polarization defines the front-back axis for the cells. Together with the up-down axis, which is orthogonal to substrate, the cells are able to establish a consistent LR axis in the regions close to the boundary. Consistently, biased alignment and directional migration are mostly observed at geometrical boundaries. Muscle cells exhibiting the CCW alignment (Figs. 2E–F) migrate in a CCW fashion on the outer ring, and in a CW direction on the inner ring. The seemingly opposite directional motion on appositional boundaries is consistent from a cell’s perspective, defined as leftward bias. Similarly, hUVECs have the ‘rightward’ bias.

Actin function plays an important role in chiral morphogenesis on micropatterned surfaces. The treatment of cells by very low concentrations of actin-interfering drugs, the leftward-bias migration of muscle cells was reversed to a rightward bias, along with the reversal of the CCW cell alignment. The tubulin-interfering agents do not have similar effects on the cells. Interestingly, such cellular responses to the drugs are similar to those of snail embryos at 4-cell and 8-cell stages.15

Taken together, these results suggest that most, if not all, cells are intrinsically chiral. With definite polarization on boundaries and intrinsic cellular machinery that tells left from right, such intrinsic chirality can be translated into multicellular LR asymmetry through biased cell alignment and directional motion.

Relevance to Embryonic Development

The establishment of LR asymmetry on micropatterns has great similarity with embryonic development, with respect to the initiation of directional motion and the role of actin cytoskeleton. Directional cell motion is also critical for embryonic development of chicken. The leftward movement of cells around the Hensen’s node, expressing sonic hedgehog and fibroblast growth factor 8, is thought to be responsible for establishing the asymmetric expression patterns and subsequent anatomical asymmetries of organs.16

Actin plays a major role in establishing LR asymmetry as for micropatterned morphogenesis. In the Xenopus egg, which is radially symmetric, a large-scale invariant chiral torsion can be induced with an actin-interfering drug, indicating the existence of maternally inherited, microfilament-dependent, chiral cellular structure.17 The proteins that are chiral targets (eg, Polaris and INV) are also found to distribute asymmetrically.
at very early stages of embryonic development of several different animals, depending on the microtubule and actin cytoskeletal organization. For LR patterning in C. elegans, the regulation of actin associated cortical contractility is responsible for LR asymmetric protrusions and handed collective movement of paired sister cells, leading to chiral morphogenesis. Recently, it has been shown that myosin ID switches the LR polarity in planar cell-shape chirality and DE-cadherin and leads to the left-handed rotation of the Drosophila embryonic hindgut. Thus, micropatterned chiral morphogenesis may closely resemble the early LR symmetry breaking during embryo development.

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