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Feeling the right force: How to contextualize the cell mechanical behavior in physiologic turnover and pathologic evolution of the cardiovascular system

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ABSTRACT

Although traditionally linked to the physiology of tissues in 'motion', the ability of the cells to transduce external forces into coordinated gene expression programs is emerging as an integral component of the fundamental structural organization of multicellular organisms with consequences for cell differentiation even from the beginning of embryonic development. The ability of the cells to 'feel' the surrounding mechanical environment, even in the absence of tissue motion, is then translated into 'positional' or 'social' sensing that instructs, before the organ renewal, the correct patterning of the embryos. In the present review, we will highlight how these basic concepts, emerging from the employment of novel cell engineering tools, can be linked to pathophysiology of the cardiovascular system, and may contribute to understanding the molecular bases of some of the major cardiovascular diseases like heart failure, heart valve stenosis and failure of the venous aorto-coronary bypass.

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1. Introduction

In 1981, a report from Johnson and Ziomek (1981) introduced, for the first time, a geometrical concept in the regulation of one of the most remarkable events in pre-implantation developmental life: the correct recognition of the cell identity and determination of their fate. Using a simple fluorescent lectin staining, they observed that cells in the developing morulae had an irreversibly established polarity starting at the eight-cell stage, which depended upon cell contacts and culminated in the blastocyst formation, the event accompanying the first lineage divergence between embryonic and extra-embryonic cells. They predicted that the specific geometrical arrangement of the cells, and thus their polarity, had to be linked to position-dependent gene expression control acting in the segregation between the first extra-embryonic lineage (the trophectoderm) and the pluripotent cells of the inner cell mass. After 35 years, the modern biology translation of this concept is more than timely, given the recent discovery that the activation of 'position sensing' effectors by a higher cytoskeleton tensioning in outer *vs.* inner cells acts at early stages of pre-implantation embryos development as the crucial determinant of trophectoderm differentiation *vs.* naïve pluripotent state in the outer *vs.* the inner forming blastocyst compartments (Boroviak & Nichols, 2014).

Position sensing is only one among the various ways cells can interpret mechanical cues from the environment. In fact, static and dynamic

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motion sensing and force transduction from/to the cells to/from the surrounding extracellular matrix have been recognized as potent morphogenetic signals. But is it cellular mechanosensing a driving force for correct cellular patterning in the cardiovascular system? And is it possible to conceive some of the crucial cellular events involved in cardiovascular diseases as 'misinterpretations' of the mechanical environment? In the present contribution we will attempt to discuss how the modern technology allows to decrypt cellular mechanosensing as a deterministic cue that might link the changes in tissue microenvironment and the evolution toward pathologic phenotypes of (progenitor) cells naturally programmed to cardiovascular renewal and homeostasis.

2. Feeling the right force at micrometric ranges: decrypting the role of cell-to-cell contacts and of extracellular matrix physical properties in dynamic control of cell fate

2.1. Cell autonomous responses to geometric cues

Mechanical feeling of the environment and the physical perception of surrounding cells involves a bi-directional short-range exchange of information. An outside-in mechanism is responsible for intracellular transduction of extracellular mechanical forces (under steady conditions or dynamic motion) and/or for instructing positional information regulating cell fate inside a growing cell aggregate; an inside-out response ensures adaptation of the cells and remodeling of the surrounding environment. Decryption of these pathways in cell culture plates has been impossible until a decade ago, either for the high level of mechanical stress cells receive when in contact with conventional tissue culture plastics and the lack of specific constraints in an organized and polarized tissues geometry. The employment of fabrication methods directly derived from other engineering areas (e.g. microelectronics) such as soft lithography, micro-contact printing and dip pen nanolithography, has changed this scenario by setting up techniques to geometrically control the tissue culture environment or by mimicking the effect of the surrounding matrix in the third dimension. To this purpose, geometrically-defined cell attachment areas (Vunjak-Novakovic, 2008), microwells tailored to culture controlled number of cells (Giobbe et al., 2012), functionalized 'smart' culture surfaces displaying optimal presentation of growth factors (Alberti et al., 2008; Pompe et al., 2010) have been manufactured to perform experiments, in single cells or multicellular aggregate setups, to unravel the intricate relationships between mechanical perception of the environment and the interactions with biohumoral signaling.

The striking results obtained employing these systems have allowed to put into a direct relationship the dynamics of the cytoskeleton polymerization/depolymerization with cell fate, thus establishing a working model for understanding the short range mechanotransduction effects as a continuum linking the tissue mechanical properties (*e.g.* elasticity, presence of specific ECM composition/arrangement) with gene expression. In particular, it was found that geometric confinement of single cells into spaces with a higher or lower cytoskeleton polymerization (*e.g.* quantifiable by formation of actin stress fiber) may have profound effects on alternative differentiation of cells with mesenchymal characteristics into osteogenic (high cytoskeleton polymerization; high cell spreading) or adipogenic (low cytoskeleton polymerization; low cell spreading) phenotypes (McBeath et al., 2004). The machinery responsible for actin fiber dynamics has been then put under scrutiny with evidences that interfering with the stress fibers formation depending on the RhoA GTP-ase/ROCK intracellular pathway can reverse the geometric confinement-dependent differentiation (McBeath et al., 2004), alter the cellular responses to cytokine signaling (Gao et al., 2010), and modify the cellular global gene expression profile (Kilian et al., 2010), thus clearly demonstrating the intricate crosstalk between mechanosensing-driven cellular responses and bio-humoral control of tissues homeostasis.

While the micro-structured cell adhesion substrates have offered a powerful method to dissect fundamental relationships between the forces engaging the cellular cytoskeleton and cell fate by connecting in a quantitative manner the discrete cell tensioning with gene expression programming, the evolution of surface patterning and functionalization to the nanoscale allows to undercover the fine regulation of cellular mechanosensing by decrypting the role of 'local' structuring of the cytoskeleton and the mechanical characteristics of the surrounding environment with a higher degree of refinement. This is essential to understand how the intracellular machinery reacts to engagement of the cytoskeleton by subtle differences in mechanical characteristics of the surrounding matrix, thereby enabling to decrypt the cell polarization requirements to regulate cell fate inside multicellular contexts. In a remarkable example, Coyer et al. employed a nano-patterning surface functionalization method to structure nanoscale adhesive 'pads' with fibronectin and assessed the RGD-dependent focal adhesion contacts assembly in relationships with adhesive forces generation by the cells (Coyer et al., 2012). The results showed that by increasing the dimensions of the nano-patterned areas supporting formation of focal contacts, non-linear increases in cell adhesion forces were observed, also involving differential recruitment of stress fibers docking molecules such as vinculin and talin and promoting discrete actin stress fiber formation. In two other examples, finally, nanoprinting of ECM components and nanogrooves manufacturing were employed to offer chemically and physically complex adhesion surfaces to cells for an understanding of cell differentiation cues (Iannone et al., 2015; Amin et al., 2016). Altogether, these results suggest the existence of threshold levels of integrin binding to the extracellular domains necessary to transduce sufficient levels of intracellular mechanical forces able to allow cells to attach to ECM adhesion domains. These evidences also indicate that chemical and physical nano-structuration of culture environment is likely a crucial strategy to finely tune the cell phenotype and achieve a highly coordinated cell regulation in future tissue engineering scenarios.

2.2. Geometric regulation of cell fate in multi-cellular colonies — stepping into 'community' effects discriminating cellular identity and functions in tissue contexts

In a position article appeared in 2011, the understanding of the cellular 'functional appearance' (or 'functiotype') (Roeder et al., 2011) in tissues and their stem cell niches has been discussed as a future key for dissecting the cellular phenotype resulting from the combined effects of humoral and metabolic signals with force-transduction machinery. The engineering of culture systems tailored to decipher the intercellular signaling in geometric-dependent manners have thus helped to find evolved interpretations of the 'community effect', a concept used in classical embryology to explain the mutual phenotype induction acting at a short ranges between cells belonging to different embryonic fields (Gurdon, 1988).

Tools analogous to those described in the previous chapter have been therefore very useful to manufacture 2D culture surfaces instructing topology-related cell differentiation into multicellular aggregates. The goal of these studies was to determine whether position-dependent cell responses occur depending on their positioning in the patterns and whether the geometry of the patterns themselves promotes different behavior of cells confined into their boundaries in a similar way to how the cell positioning in the embryo establishes the lineage of the cells.

In a first example, the combination of geometric patterning and electrodes allowing electrostatic trapping into a microfluidics circuit allowed to immobilize onto geometrically defined surfaces cells that were forced to expose discrete levels of their cell membranes to physical contacts with neighboring cells (Gray et al., 2008). By controlling cell contact of normal cells with cells genetically modified for the intracellular transduction force machinery dependent on the cytoskeleton tensioning (e.g. RhoA), the authors were able to establish a