The 676th Kitasato Medical Society Invitational Academic Lecture Series Abstract

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Challenges in cardiovascular tissue engineering

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Surgical intervention requiring prosthetic implants continues to be critical in the treatment of many adult patients, and despite significant therapeutic advances in cardiovascular disease, heart transplant remains the only definitive treatment for end-stage heart failure. In addition, congenital heart disease is a leading cause of neonatal death, and surgical reconstruction is often unavoidable in this patient population. Also, there are significant challenges using existing prosthetic materials, such as calcification, host rejection, infection, somatic overgrowth and durability issues.

Cardiovascular tissue engineering has the potential to generate an unlimited amount of functional tissue, and has the ability to grow and remodel, thus improving the quality of life of many patients. There are many fields and approaches in tissue engineering, my/our research focuses on two specific areas: 1) tissue engineered vascular grafts (TEVGs) using cell-free biodegradable scaffolds, and 2) 3D-bioprinted human cardiac patches using induced pluripotent stem cells (iPSCs).

Clinical studies have established that TEVGs are safe and effective to use in the low-pressure venous circulation system of pediatric patients undergoing extracardiac total cavopulmonary connection procedures. However, current TEVGs do not directly address the diverse anatomic and physiologic requirements of individual patients. Recent imaging technologies, such as CT and MRI, are now able to provide surgeons with detailed 3-D views of cardiovascular anatomies. Leveraging these advances in technologies, we have developed a novel patient-specific nanofiber TEVG using modern imaging/3D-printing/electrospinning methods. We have successfully implanted these new constructs in a large animal model. In addition, small-diameter (less than 6 mm) TEVGs have been developed to withstand the high pressures of the arterial circulation. However, arterial TEVGs have not yet been successfully translated into clinical therapy because slow degrading materials have limited cell infiltration and neotissue formation, and display localized calcification in the long term. Thus, our current research effort into fabricated arterial TEVGs is focused on finding the ideal blend of materials and manufacturing techniques.

At the same time, we are pursuing a novel method to create scaffold-free cardiac muscle tissue using a 3D-bioprinting platform that assembles multicellular spheroids consisting of iPSC-derived cardiomyocytes, endothelial cells and fibroblasts, on a "Kenzan" needle array. While the conventional paradigm for cardiac tissue engineering is to combine cells suitable for cardiac therapy with a 3D scaffold, we have demonstrated that 3D-bioprinting of cardiac tissue without the use of biomaterial is feasible and 3D-bioprinted cardiac patches exhibit spontaneous beating, mechanical integrity, electrical integration of component cardiospheres and ventricular myocyte-like cellular electrophysiological properties. As a result, 3D-bioprinting of cardiac tissue is a highly promising area of study within the field of cardiac tissue engineering.

In conclusion, cardiovascular tissue engineering is a vibrant area of research with applications such as TEVGs and human cardiac patches. Despite the promising potential of tissue engineering, the precise mechanisms including cardiovascular remodeling have yet to be elucidated. A multidisciplinary approach involving biologists, biomedical engineers and clinicians is needed to fully understand and bring these new technologies to clinical practice.

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Tumor associated macrophages: from mechanism to therapy

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There is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression.[1,2] Macrophages enhance malignancy at the primary site by stimulating angiogenesis, inducing tumor cell migration, invasion and intravasation and by suppressing anti-tumor immunity.[3] At metastatic sites, macrophages promote tumor cell extravasation, survival, and subsequent growth.[2] Each of these pro-tumoral activities is promoted by a sub-population of macrophages that expresses canonical macrophage markers but also has unique properties as exemplified by surface markers and transcriptional profiles.

This macrophage diversity is exemplified at the metastatic site where a population of macrophages termed, metastasis associated macrophages (MAMs), are recruited to the extravasating tumor cells. These MAMs have a different phenotype from macrophages in the primary tumor in terms of cell surface markers. They are derived from circulating inflammatory monocytes (IM) that are recruited to the metastatic site through a CCL2-CCR2 signaling pathway. Inhibition of this recruitment using genetic intervention or by neutralizing antibodies to CCL2 reduces metastatic tumor cell seeding, tumor cell survival and persistent growth and extends survival of mice.[4]

Immediately upon recruitment the IMs differentiate through a number of steps to become MAMs each of which is characterized by the up-regulation of different cell surface signaling receptors or receptor ligands. For example monocyte retention requires a chemokine cascade with CCL2 inducing CCL3 with each chemokine acting sequentially.[5] Furthermore, upon MAM differentiation there is up regulation of VEGFR1 whose function is essential to their metastasis promoting activity and which links VEGF signaling with an inflammatory cascade.[6] We have defined the requirements for many of these signaling pathways in metastatic tumor cell dissemination and suggest that such pathways may provide novel therapeutic targets to treat metastatic disease.

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Mechanisms of blood vessel leakiness in inflammation and cancer

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Blood vessel leakiness is a well-documented feature of inflammation and cancer. Plasma leakage under these conditions results from alterations in endothelial barrier function. The cellular basis of this alteration is attributed to the formation of gaps between endothelial cells. When initially described in 1961, endothelial gaps were reported to occur in postcapillary venules after exposure to histamine, serotonin, or bradykinin. Subsequent studies of leaky blood vessels in inflammation revealed that the gaps are small, having a mean diameter of only 0.3 μm (range, 0.2－1.6 μm). Blood vessel leakage in tumors, which facilitates angiogenesis and delivery of cancer diagnostics and therapeutics, results from larger gaps (mean, 1.7 μm; range 0.3－4.7 μm), and other endothelial defects. Additional cellular processes, including transcytosis, transcellular holes, and vesiculovacuolar organelles (VVOs), have also been implicated, but evidence for endothelial gaps as routes of plasma leakage remains solid. Although many cytokines, including VEGF and TNF-alpha, can trigger plasma leakage, few agents have anti-leakage effects and even fewer act selectively on endothelial cells. The angiopoietin/Tie family of ligands and receptors are exceptions. Angiopoietin-1 (Ang1, Angpt-1), which activates Tie2 receptor signaling in endothelial cells, suppresses gap formation and reduces leakage induced a wide range of mediators. These actions are strongly influenced by angiopoietin-2 (Ang2, Angpt-2). Unlike Ang1, Ang2 has context-dependent effects on Tie2 signaling. Ang2 promotes Tie2 activation and decreases leakage in some conditions, but paradoxically, competes with Ang1, inhibits Tie2, and increases leakage under other conditions. In studies done to reconcile this paradox, we found that Ang2 acts as a Tie2 agonist in normal mice, where it promotes Tie2 activation and vascular stability. By contrast, in inflammation, Ang2 acts as a Tie2 antagonist, increases gap formation and leakiness, and also increases Ang2 expression. These actions of Ang2 are governed by Tie1 receptors, which are abundant in endothelial cells under normal conditions. Tie1 is an orphan receptor that does not bind angiopoietins but undergoes rapid inactivation by ectodomain shedding in inflammation, and acts as a switch for the agonist/antagonist actions of Ang2. High levels of soluble Tie1 ectodomain and Ang2 are found in blood of critically ill patients and are correlated with poor outcome. In tumors, VEGF destabilizes the vasculature and increases leakiness; Ang2 amplifies these actions. Inhibition of VEGF and Ang2 normalizes tumor vessels and reduces leakage. Together, the evidence shows that in health, Ang1 is dominant, maintains Tie2 activation, suppresses endothelial gap formation, and preserves vascular stability. Ang2 has similar but weaker actions under these conditions. However, in inflammation, when Tie1 is inactivated by ectodomain shedding, Ang2 acts as a Tie2 antagonist that dominates the actions of Ang1. Inactivation of Tie2 promotes endothelial gap formation and vascular leakiness. Elevated Ang2 and soluble Tie1 in blood are biomarkers of endothelial injury. In cancer, VEGF and Ang2 promote vascular instability and leakage. Although angiopoietin/Tie receptor signaling is more complex in tumors than in inflammation, angiopoietins and Tie receptors are promising diagnostic and therapeutic targets in both conditions.