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A lymphatic co-culture model for personalized cancer medicine

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Head and neck squamous carcinoma (HNSCC) arises from the mucosal epithelium in the oral cavity, throat, and larynx, and the majority of HNSCC patients develop lymph node metastasis which is correlated with poor prognoses and increased risk of distant metastasis [1]. Undoubtedly, there is an urgent need to decipher biological and biophysical factors underlying the lymphatic metastasis to prevent tumor spread. Tumor lymphangiogenesis involves remodeling of pre-existing lymphatic vessels to be more permeable as well as formation of new lymphatic vessels from the existing vessels, which are considered as a favor entry of cancer cells into the lymphatic system [2]. There is also growing evidence highlighting tumor microenvironment (TME) contribution to HNSCC progression. For example, TME in HNSCC is characterized by highly complex and heterogeneous stroma consisting of cancer associated fibroblasts (CAFs) that have been shown to induce lymph node metastasis in HNSCC [3]. To understand crosstalk between CAFs and lymph node metastasis, it is critical to create model systems that allow to dissect the molecular pathways between them, and to identify individualized targets to treat patients when taking the HNSCC tumor heterogeneity into consideration as the heterogeneity leads to inconsistencies in patients' drug responses.

In this issue of EBioMedicine, Lugo-Cintrón and colleagues investigated the roles of HNSCC patient tumor derived fibroblasts in lymphangiogenesis *in vitro* using a three-dimensional (3D) microfluidic system [4]. In general, 3D microfluidic systems consist of multiple compartments and cells embedded in extracellular matrix (ECM) with biophysical and biochemical cues mimicking multicellular tumor architecture, bridging the gap between conventional 2D cell culture and animal models [5,6]. In their study, Lugo-Cintrón and colleagues investigated fibroblast-induced alterations in lymphatic endothelial gene expression using their lymphatic coculture model to understand the heterogeneity of HNSCC and predict potentially effective treatments for inhibiting tumor lymphangiogenesis [4]. They utilized previously developed 3D microfluidic system with a single hollow cylindrical channel embedded in an extracellular matrix [7,8]. Primary lymphatic endothelial cells (LECs) were seeded in the channel surrounded by the extracellular matrix containing HNSCC patient-derived fibroblasts. In the coculture system, authors observed that patient-derived fibroblasts increased lymphatic sprouting and vessel permeability compared to the LEC monoculture. They followed up with gene expression analyses in LECs cocultured with patient's derived fibroblasts. Interestingly, they found two candidate genes upregulated in the coculture with patient's fibroblasts: insulin like growth factor 1 (IGF1) and integrin beta 3 (ITGB3). It seems that those genes might be critical for pro-lymphangiogenic "behaviors" of lymphatic vessels. They also found thrombospondin 1 (THBS1), an anti-lymphangiogenic and anti-angiogenic factor, was downregulated in the coculture with patient's fibroblasts, which showed elongated sprouts in their coculture model. Finally, they showed that targeting ITGB3 reduced lymphatic sprout lengths, the numbers of sprouting, and permeability. Combinatorial treatments with those targets could be beneficial to inhibit lymphatic tumor metastasis in HNSCC.

Microfluidic technologies hold a potential for pre-clinical cancer drug testing [5]. Traditional animal models have contributed to cancer drug development. However, researchers often cannot predict how the drug candidates selected based on animal studies will be effective in human trials, given the genetic discrepancies between humans and the experimental animals [9]. Nowadays, researchers are creating more complex systems resembling 3D multicellular structures, physical motions, forces, and fluid flow using devices containing human primary or/and inducible pluripotent stem cell (iPSC) derived organoids [10], which would better recapitulate multifactorial tumor pathologies. In summary, Lugo-Cintrón and coworkers developed a model mimicking the head and neck tumor microenvironment composed of lymphatic vessels and cancer patient-derived fibroblasts. Their model demonstrated biological crosstalk between lymphatics and tumor-derived fibroblasts and predicted potential targets for tumor lymphangiogenesis. In addition to LECs and fibroblasts, immune cells and blood endothelial components may account significantly for the TME in HNSCC, given blood vessels are the main route of lymphocyte infiltration to tumors and the targets the authors identified, such as IGF1, ITGB3, and THBS1, also affect tumor angiogenesis and blood vessel function. On another note, redesigning their

microfluidic system towards more high-throughput models would facilitate testing patient samples and targets efficiently.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

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