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Integrative multi-omics approaches for discovery of new drug targets for cardiovascular disease

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In efforts to identify cell type-specific causal processes in vascular diseases, our research communities have rapidly evolved from use of a single or a few pre-selected markers to non-biased tissue and single-cell analyses. Such analyses of human and mouse tissues have provided insights into the amazing diversity of cell populations in vascular diseases and atherosclerotic lesions.[1] Non-biased “omics” approaches, including transcriptomics, proteomics and metabolomics, are now increasingly used in combination with single-cell technologies and spatial profiling to investigate vascular diseases.

A general model for the discovery and validation of novel pathways and drug targets using integration of non-biased -omics and single-cell approaches is described in Figure 1. The discovery phase compares human diseased tissues with healthy control tissues and tissues from animal models of the disease. Because differences in cell type composition of diseased and normal tissues can introduce bias when using bulk tissue profiling, singe-cell non-biased -omic strategies are most informative. The integrative computational phase makes use of experimental data and publicly available resources on human disease, cross-species data, and cross-platform data. For example, cross-species reference-based analyses of single cell data[2, 3] give a framework for integration of experimental and genetic manipulations in model systems to provide greater mechanistic insights into human disease. Similarly, cross-platform integration of public human genetic data facilitates causal and directional inference for genes, proteins and metabolites in a cell-specific context.[4] The molecular functions of novel targets and pathways as well as computationally predicted master regulators[3, 6] can be investigated in isolated cells and using gene-editing technologies and human induced pluripotent stem cell systems. Validation is critical to prove causality and significance of

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identified pathways and specific targets, first in animal models and then, through clinical translation, in cogent clinical trials. This strategy can ultimately leverage precision medicine principles and targeted therapeutics, which allow treatment of patients according to their specific set of risk factors and molecular and cellular disruptions.

Using elements of the approach in Figure 1, in this issue of *Circulation* Decano and colleagues describe a proteomics-driven systems approach to identify drug target candidates for the treatment of vein graft failure[5]. Vein grafts are used to treat peripheral artery disease and as coronary artery bypass grafts. However, many of these grafts become occluded and fail with time, developing lesions in part similar to those in atherosclerotic arteries. There are currently no effective treatments available for prevention of vein graft failure, as statins and other drugs that reduce atherosclerotic cardiovascular disease (CVD), do not appear to be effective.

Decano et al.[5] reasoned that a non-biased approach would be most useful for identification of potential novel drug targets. In a series of elegant mouse studies, the authors first utilized global tissue proteomics to analyze the inferior vena cava from donor mice sutured to the carotid artery to create vein grafts in a small number of fat-fed LDL receptor-deficient (*Ldlr*$^{-/-}$) recipient mice and wildtype mice fed a standard rodent diet (discovery phase, Figure 1). Vein grafts in fat-fed *Ldlr*$^{-/-}$ mice developed lesions containing smooth muscle cells and macrophages and had a proteome composition markedly different from that of the control undisturbed vena cava. Proteomics performed at several different time-points up to 4 weeks after vein grafting identified 30 protein clusters regulated in temporally distinct fashions in vein grafts versus the undisturbed vena cava. Because single-cell proteomics was not used in the discovery phase, a significant portion of the identified changes in tissue proteome was likely due to differences in tissue cell type composition. Caution is also warranted given the lack of primary non-biased discovery in human diseased tissues, particularly in considering the relevance of discoveries in a severely hyperlipidemic mouse model to the human graft disease, which often emerges despite low circulating lipoproteins and aggressive treatment of hypercholesterolemia.

Pathway networks were then constructed, and the most central proteins in each pathway were computed. An overall network associated with inflammation, response to toll-like receptor (TLR) ligands, and extracellular protein remodeling was amplified in vein grafts versus non-grafted veins. Further, an early (1–3 days) response network in vein grafts was associated with cytoskeletal reorganization and blood coagulation, whereas a late (14–28 days) network associated primarily with chemotaxis. Conversely, a network of proteins involved in metabolism was amplified in normal versus grafted veins. Next, network proximity analysis, integrating data from several human vascular diseases, suggested a link between the mouse vein graft proteome and human arteriovenous fistula disease. Ultimately, experimental data from human vein graft disease would provide greater confidence of target discoveries. Large-scale genetic data, which could provide strong support for causality and directional effects of candidates, are not yet available for human vein graft disease.

Peroxisome proliferator-activated receptor-α (PPARα), a ligand-activated transcription factor known to enhance fatty acid beta-oxidation, and a central protein in the network of

proteins enhanced in undisturbed veins, as compared with vein grafts, was selected for further analysis (validation phase, Figure 1). In mouse experiments, silencing macrophage PPARα by nanoparticle delivery of siRNA worsened lesions in vein grafts, whereas treatment with the selective PPARα activator pemafibrate[7] slowed onset of vein graft lesion development, reduced lesion accumulation of macrophages, and also protected against detrimental changes in an arteriovenous fistula mouse model.

Next, the authors performed a series of single-cell RNA-sequencing and metabolomics studies in isolated human macrophages stimulated with the TLR4 ligand lipopolysaccharide (LPS) and treated with pemafibrate. These studies suggested that pemafibrate prevents some of the inflammatory effects of LPS in a subpopulation of human macrophages, while increasing genes related to fatty acid oxidation. The metabolomics showed that PPARα activation indeed shifts metabolism from glycolysis to oxidative respiration. Finally, Decano et al. performed a directed regulatory network analysis to investigate the relevance of the *in vitro*-generated metabolomics data to the vein graft mouse model. These results, based on a small number of mice, appeared to support a direct role of PPARα in vein graft homeostasis.

While the study by Decano and colleagues is an excellent example of use of a non-biased approach for identification of protein targets involved in vein graft disease, further studies will be needed to achieve the goal of using this systems approach for identification of targets in a truly non-biased and human disease-relevant manner. While selection of PPARα for validation studies, rather than one of the more novel proteins identified, did provide important proof-of-concept, PPARα activation by pemafibrate has previously been shown to suppress atherosclerosis and vascular response to injury,[8, 9] and to increase fatty acid oxidation and suppress inflammatory activation in macrophages.[10] Thus, the selection of PPARα as the target for follow-up studies, as well as the selection of cell type (i.e., macrophages rather smooth muscle cell-derived cell types), the lack of primary discovery in human tissues, and the severe hyperlipidemia of the mouse model introduce biases and limitations to the approach used.

A novel aspect of the study is the demonstration that pemafibrate prevents vein graft disease and arteriovenous fistula pathology in mice. In support, gemfibrozil, an older fibrate, reduced the number of new lesions in venous aortocoronary bypass grafts in a small clinical trial of men with low HDL-cholesterol.[11] The large PROMINENT trial (NCT03071692) should report in 2022 and will reveal if pemafibrate will prevent cardiovascular outcomes in subjects with type 2 diabetes and elevated plasma triglycerides.[12] Pemafibrate has a higher potency and fewer off-target effects than previously tested fibrates, which have largely failed to prevent CVD.[13] Decano et al. selected a dose (0.2 mg/kg body weight/day) of pemafibrate to not lower plasma triglycerides, although this dose did have a triglyceride-lowering effect under fasting conditions. Furthermore, this dose is higher than that used in PROMINENT (0.2 mg twice/day), a dose that lowers triglycerides by approximately 50% in humans.[14] It is therefore possible that the beneficial effects of pemafibrate on vein graft lesions in the mice might have been due in part to modulation of circulating lipid species, or to indirect effects of pemafibrate in other tissues, such as increased hepatic expression of the anti-atherogenic FGF21.[15]

In summary, Decano et al. provide an interesting proof-of-principle supporting the concept that drug targets effective in preventing complex CVD, such as vein graft disease, can be identified by integrative systems approaches. Will pemafibrate be effective in preventing vein graft disease in humans? While we await the results of the PROMINENT trial and before a placebo-controlled clinical trial of pemafibrate is indicated for vein graft disease, much can be done to build on the current work and to delve more deeply into a fully integrated systems approach (Figure 1) to identify novel molecular and cellular targets for vein graft disease in humans.

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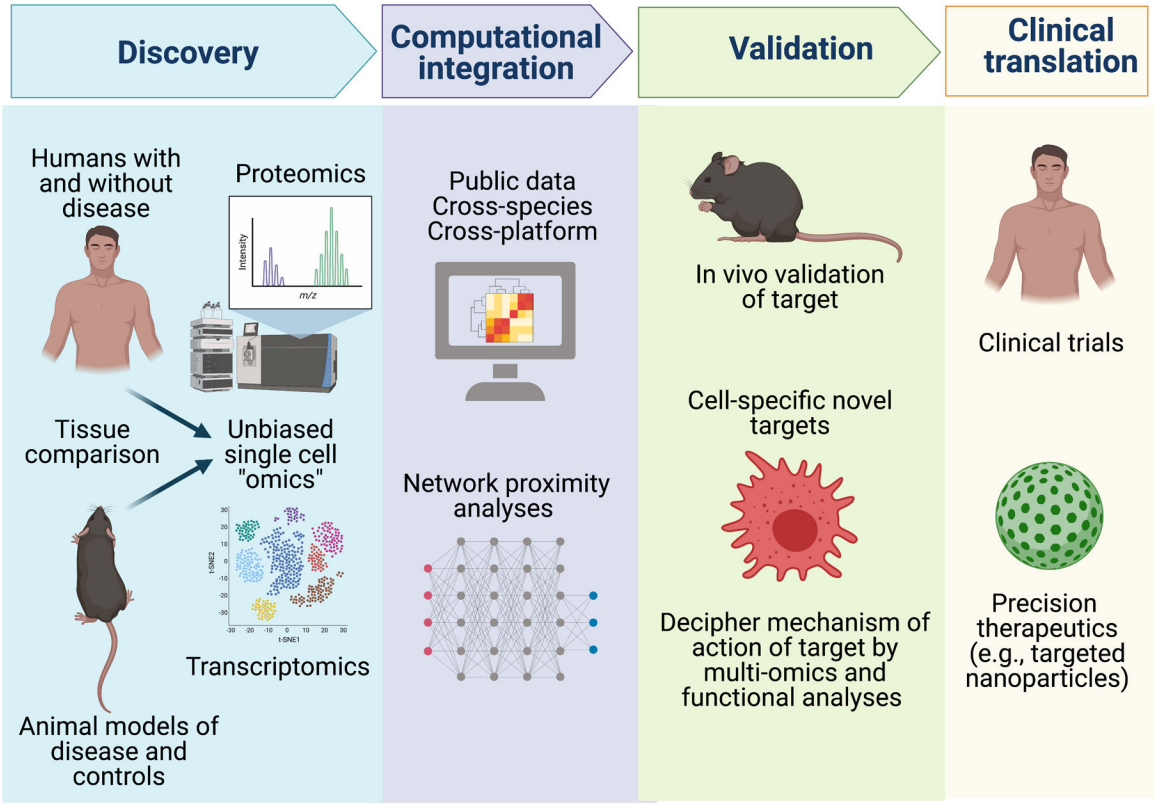

Figure 1. Model for discovery and validation of novel drug targets for complex diseases using integrative single-cell non-biased -omics.

A discovery phase using both human disease and animal model single cell -omics profiling is recommended. Integrative computational methods are applied to cross-species and cross-platform data to identify and prioritize master regulatory and druggable targets of greatest importance in human disease. Experimental functional studies in animal models of disease and in disease-relevant human cells, including gene-manipulation in human induced pluripotent stems cells, can define precise molecular and cellular targets for translation via clinical trials and precision therapeutic strategies. Figure created with BioRender.com.