Identification of organ-specific transcriptomic shifts in the vasculature during systemic inflammation using TrendCatcher

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Keywords:  
Endothelial cells  
Endothelial heterogeneity  
RNA-seq  
Differential expression  
Dynamic programming  
Vascular biology  
Inflammation  
Transcription  
Translation  
Systems biology  
Pathway analysis  
Brain endothelium  
Heart endothelium  
Lung endothelium
Abstract
A recent analysis of endothelial cell (EC) gene expression suggests that ECs of distinct organs vary in their gene expression profiles and respond distinctly to the systemic inflammatory stimulus of endotoxemia (Jambusaria et al., 2020). There is a need to systematically define tissue-specific gene expression dynamics in response to inflammation but such an analysis is in part limited by the availability of appropriate algorithms to analyze differential expression across a time course. Here, we present TrendCatcher (https://jaleesr.github.io/TrendCatcher_1.0.0/), an R package designed for time course RNA-seq data analysis which identifies distinct dynamic transcriptional programs. When applied to ECs, we observed that approximately 85-95% of EC genes in all three organs followed a biphasic response following endotoxemia. The rapid upregulation of innate immune response and bacterial response genes occurred within 6 hours in all three vascular beds but the subsequent upregulation of reparative EC mitosis and cell cycle genes occurred most rapidly in lung ECs (24-48h) and was most delayed in cardiac ECs (72h-168h). The distinct kinetics of EC inflammatory injury and regeneration identify vascular-bed specific temporal windows for targeted therapeutics.
Introduction

Endothelial cells (ECs) form the inner cellular lining of all blood vessels and establish a barrier between the circulating blood and the underlying parenchyma (Aird, 2012; Bautch, 2011). ECs perform a wide range of critical functions in many physiological processes, such as maintaining intravascular homeostasis, trafficking nutrients and waste between blood and the parenchymal tissues, as well as initiating and regulating immune responses (Liao, 2013). Despite sharing numerous essential roles in physiology, ECs exhibit distinct molecular and functional properties across different tissue types and vascular locations (Nolan et al., 2013; Potente & Mäkinen, 2017). EC heterogeneity has been validated and characterized in both within-organ and organ-specific scenarios. For example, studies focused on characterizing EC heterogeneity within single organs (Sabbagh et al., 2018; Su et al., 2018; Vanlandewijck et al., 2018) have revealed that local cues from the tissue microenvironment, such as metabolites, cell-cell interactions, and cell-matrix interactions, are critical for EC heterogeneity (Lukowski et al., 2019; Potente & Mäkinen, 2017; N. Yucel et al., 2020). The heterogeneity of the microvascular endothelium across organs is apparent in the distinct gene expression profiles of ECs from each major organ (Jambusaria et al., 2020; Kalucka et al., 2020; Nolan et al., 2013; Paik et al., 2020).

To investigate the transcriptomic basis of EC heterogeneity, unbiased transcriptomic profiling is essential for providing precise insights into the underpinning molecular mechanisms. Bulk RNA sequencing (RNA-seq) and single-cell RNA sequencing (scRNA-seq) provide viable approaches to study global gene expression. Studies using scRNA-seq have characterized transcriptomic signatures of distinct EC subpopulations and the imprinted transcriptome (Kalucka et al., 2020; Paik et al., 2020). However, the isolation of defined cell populations from their physiological tissue context is required to prepare single-cell suspensions (Eberwine, Sul, Bartfai, & Kim, 2014). Disaggregation of ECs for single-cell analysis or for flow cytometry cell sorting results in the loss of homeostatic outside-in signaling cues from neighboring cells and the extracellular matrix, and could thus alter gene expression profiles (Haimon et al., 2018; van den
Brink et al., 2017). The complementary approach of RiboTag-based translatome profiling avoids cell removal from their native niche and enables the direct isolation of tissue-specific ribosome-attached mRNAs without cell dissociation (Cleuren et al., 2019; Sanz et al., 2009). More importantly, this genetic approach allows the direct probing of EC heterogeneity in situ, so it offers the recovery and sequence analysis to evaluate the program directly from intact organs (Cleuren et al., 2019). A recent study of the translatome in the cardiac endothelium demonstrated that the tissue-specific endothelial gene expression profile is a reflection of signature open chromatin regions within the cardiac endothelium, which are lost once the cardiac endothelium is removed from its native environment and cultured (N. Yucel et al., 2020). Furthermore, RiboTag complements conventional global bulk RNA-seq and scRNA-seq EC transcriptomic analysis by focusing on mRNAs undergoing translation whereas global RNA-Seq analysis and single-cell RNA-Seq analysis provide profiles reflecting total mRNA in a cell (Jambusaria et al., 2020).

Recent studies on organ-specific EC transcriptomic profiles have focused on differences between ECs at baseline but there is a growing interest in how organ-specific EC heterogeneity changes in response to a stressor such as inflammation. Which biological responses show similar changes in magnitude and kinetics across endothelial cells from distinct organs and which stress responses demonstrate organ-specific patterns? This has been addressed in part in recent studies (Ajami et al., 2017; Chong, Yu, Brighton, Bear, & Bautch, 2017; Gunawardana et al., 2021; Tombor et al., 2021), but there is a need for a systematic and unbiased analysis of the temporal kinetics of endothelial gene expression across multiple organs. The endothelial RiboTag transcriptome data collected at 6 different time points following induction of endotoxemia from the heart, brain and lung (Jambusaria et al., 2020) is ideally suited to perform a systematic temporal analysis. Such temporal dynamic characterization would capture the timing of cellular stress response programs and provide insights into the heterogeneous organ-specific kinetics of endothelial injury and repair.
Analysis of time-course RNA-seq data represents a computational challenge. Currently, there are mainly two strategies to address these challenges. One strategy treats sampling time points as categorical variables and is based on generalized linear models (GLMs). For example, the software packages DESeq2 (Love, Huber, & Anders, 2014), edgeR (McCarthy, Chen, & Smyth, 2012), and limma (Ritchie et al., 2015) use this strategy. These methods focus on the magnitude of change instead of the time order of gene expression, and may also suffer from a relative loss of statistical testing power, especially if many time points are assessed (Fischer, Theis, & Yosef, 2018). A complementary strategy is to treat time as a continuous variable and fit the time expression data into a spline-like model. Such methods include DESeq2Spline (DESeq2 adopted with spline model for temporal RNA-seq datasets) fitting, ImpulseDE2 (Fischer et al., 2018) and Next maSigPro (Nueda, Tarazona, & Conesa, 2014). We found the latter strategy increased the power of detecting dynamic genes, but it may suffer from a strong model assumption that oversimplifies the complicated dynamic biological scenarios, such as multiphasic responses in which gene expression patterns can oscillate between rapid increases and decreases to reflect initial responses as well as counter-regulatory compensatory responses.

In our present study, we developed TrendCatcher, a versatile R package tailored for time-course RNA-seq analysis. TrendCatcher uses a framework that combines the smooth spline ANOVA model and break point searching strategy which identifies inflection points when gene expression trends reverse. We show that TrendCatcher outperformed the leading methods for time-course RNA-Seq analysis by using simulated time course data. TrendCatcher has the unique ability to show users changes in biological pathway enrichment over time, which highlights the distinct transcriptional programs that are associated with dynamic biological processes. We applied TrendCatcher to the gene expression profiles of ECs from the brain, heart, and lung EC and identified the kinetics of injury, repair, and regeneration programs.
**Results**

TrendCatcher accurately identifies dynamic differentially expressed genes (DDEGs) in simulated datasets

First, we tested the prediction performance of the TrendCatcher platform (Figure 1) using a set of simulated time course RNA-seq datasets, because simulated data provides defined standards to assess the accuracy of novel analytical platforms. We considered a comprehensive collection of gene temporal trajectory patterns to simulate a set of realistic data with biological characteristics (Bar-Joseph, Gitter, & Simon, 2012). We embedded 1,000 constant trajectories (non-dynamic genes), 500 linear trajectories (monotonous state transition, i.e. continuously increasing or continuously decreasing gene expression levels throughout the time course), 500 impulse shaped single break point trajectories (only one temporal inflection point, i.e. up-peak-down or down-trough-up), 500 two break point trajectories and 500 three break point trajectories (more complicated dynamic response, e.g., a combination of 2 or more basic types of trajectories).

As shown in Figure 2A-C, compared to DESeq2, DESeq2Spline and ImpulseDE2, TrendCatcher achieved higher accuracy in a mixed simulated dataset. We also tested each model's performance on a varying number of time points. As shown in Figure 2D, TrendCatcher had the highest prediction accuracy across all time points tested, with accuracies of more than 75%.

We also generated 1,000 gene trajectories for each type of dynamic profile to compare each model’s performance across different trajectory types. As shown in Figure 2—figure supplement 1A-B, for basic trajectory patterns, such as linear trajectory and impulse shaped trajectory, TrendCatcher achieved accuracies of 80.8% for linear trajectory and 75.3% for impulse shaped trajectories. As shown in Figure 2—figure supplement 1C-D, 2 to 3 break points represent the combination of basic temporal trends, such as an impulse response leading to another sustained response. TrendCatcher had higher prediction accuracies of 94.2% for two break points trajectories and 91.4% for three break points trajectories and thus outperformed the other three models. Interestingly, DESeq2 and DESeq2Spline works well only when the trajectory
pattern is simple, but we observed their accuracies dropped for more general trajectory types. For example, DESeq2Spline prediction accuracy dropped to 62.46% in the three break point dynamic pattern. These results suggest that TrendCatcher can maintain its accuracy even in the setting of various complex temporal patterns (with multiple break or inflection points), which may occur in the setting of early biological responses followed by counter-regulatory adaptive responses.

**TrendCatcher identifies in situ organ-specific DDEGS and their temporal patterns composition during inflammatory injury**

We applied TrendCatcher to the previously reported RiboTag dataset of the endothelial translatome in the murine heart, brain and lungs during baseline and endotoxemia (Jambusaria et al., 2020) to identify dynamic inflammatory response genes. The RiboTag dataset (GSE136848) is an RNA-seq dataset of in situ endothelial cell response to systemic injury induced by sublethal intraperitoneal bacterial endotoxin lipopolysaccharide (LPS) at six time points and across three distinct vascular beds during homeostasis and inflammation. We analyzed the global transcriptomics profiles at 0 hour (baseline), 6 hours, 24 hours, 48 hours, 72 hours and 168 hours after LPS-injury to the brain, heart and lung. TrendCatcher identified 2,047 significantly dynamic genes in brain ECs (**Supplementary file 1**), 2,044 dynamic genes in heart ECs (**Supplementary file 2**), and 2,632 dynamic genes in lung ECs (**Supplementary file 3**). They accounted for 11.3%, 13.4% and 16.2% of the total expression profiles of brain, heart and lung, respectively, suggesting that over 80% of the expressed genes in the endothelium only fluctuate around baseline expression level in response to the profound inflammatory injury.

TrendCatcher also assigned trajectory pattern types to all dynamic genes identified from post LPS-injury, and provided hierarchical pie charts to visualize the composition of trajectory patterns across the three organs. Each dynamic gene’s trajectory pattern was characterized using a hierarchical scheme, a master-pattern type and a sub-pattern type. A master-pattern type describes the basic type of gene expression trend, for example, “up-down” represents a biphasic
response, upregulation followed by downregulation. The sub-pattern type shows the time-dependent dynamic behavior of a gene transcriptional response. For example, “0h_up_24h_down_168h” belongs to an “up-down” master-pattern type and represents peak gene upregulation at 24 hours, followed by downregulation until 168 hours.

We observed the same 8 major master-patterns across the three organs (Figure 3A) but with sub-pattern composition shifts (Figure 3—figure supplement 1-3), especially for “up-down” biphasic response with distinct upregulation time. As the inner hierarchical pie charts of Figure 3B-D show, we found over 85-95% of all the dynamic genes from brain, heart and lung ECs had a biphasic response to LPS-inflammation. The majority of the biphasic response genes were upregulated first, suggesting that there is a clear asymmetry in favor of endothelial gene upregulation instead of gene repression during inflammatory injury. We found that heart and lung ECs tended to have more early-upregulated genes at the first 6 hours (Figure 3C-D), as 51.6% of DDEGs in heart EC and 40% of DDEGs in lung ECs reached the expression peak at 6 hours. In contrast, in brain ECs only 20.3% of DDEGs reached peak expression at 6 hours. Brain EC DDEGs tended to gradually increase their expression level, such that 20% and 20.6% reached peak expression 24 hours and 48 hours after induction of inflammatory LPS-injury.

Organ-universal and organ-specific inflammatory shift using genes from the same temporal pattern

We further investigated whether dynamic genes across brain, heart and lung ECs share similar biological functions by exploring organ-universal inflammation pathways. We assessed the overlap among the three EC types for each sub-pattern gene trajectory and performed a gene ontology (GO) enrichment analysis. We found 143 “universal” (i.e. across ECs in all three organs) dynamic genes (Figure 4A, Supplementary file 4) in the early 6-hour activation sub-pattern (“0h_up_6h_down_168h”) which were enriched for self-defense-related pathways. These included response to lipopolysaccharide (19 genes), defense response to virus (27 genes) and
response to interferon-beta (14 genes), response to interferon-gamma (17 genes) (Figure 4B). These shared early activation pathways suggest an organ-universal mechanism to LPS-induced inflammatory injury, in which ECs across multiple major organs activate self-defense pathways shortly after injury, with a strong enrichment of interferon response genes.

We also assessed organ-specific pathways that were upregulated early on at 6 hours after LPS injury. Brain-specific inflammatory responses were enriched for increased neutrophil migration and regulation of angiogenesis (Figure 4C). However, pathways related to Wnt signaling were downregulated (Figure 4-figure supplement 1B), which is important because Wnt signaling is a key regulator of the integrity of the blood brain barrier (BBB) (Laksitorini, Yathindranath, Xiong, Hombach-Klonisch, & Miller, 2019). Early-peaking heart-specific EC responses were highly enriched in ribosome assembly and ribosome biogenesis processes suggesting global upregulation of protein translation (Figure 4D), but showed an early downregulation in cytokinesis pathways (Figure 4-figure supplement 1C). In lung ECs, we observed the unique upregulation of leukocyte, neutrophil, and granulocyte migration and chemotaxis, and pathways related to calcium ion homeostasis (Figure 4E), whereas endothelial cell migration gene expression was downregulated (Figure 4-figure supplement 1D).

At 24h, brain ECs upregulated ribosome biogenesis processes, mirroring the upregulation of these processes that we had observed in heart ECs at the earlier 6h time point (Figure 4-figure supplement 2B). At this time point, heart ECs concomitantly upregulated ER stress response pathways as well as proteasomal degradation pathways (Figure 4-figure supplement 2C). In lung ECs, the bulk of upregulated genes occurred at the 6h time point but we also identified selected genes peaking later at 24h, however they were also related to pro-inflammatory processes such as leukocyte migration (Figure 4-figure supplement 2D), suggesting that compared to the heart and the brain, the lung endothelium showed the most prominent sustained upregulation of pro-inflammatory genes during 6h-24h.
Time-Heatmap discloses organ-specific dynamic programming of endothelial cells

Next, we developed a Time-Heatmap function in TrendCatcher to analyze and visualize the time-dependent dynamic programming. Instead of grouping genes solely based on the trajectory sub-pattern types, which uncovers very few enriched biological pathways, TrendCatcher groups genes based on a derivative manner. It treats neighboring time points as a time window and compares each gene’s expression level within the time window to its previous break point expression level. In this way, genes sharing the same expression dynamic within a time window were grouped, independent of their whole dynamic trajectory pattern.

We first applied this approach to the lung EC dataset which has exhibited the most prominent and sustained upregulation of inflammatory genes. As shown in Figure 5, TrendCatcher captured distinct phases of lung EC inflammation: 1) inflammatory activation and injury responses, 2) EC proliferation and regeneration as well as 3) EC migration and angiogenesis. The enrichment of host-defense pathways, such as innate immune response and response to bacterium, were strongly activated within the 0h-6h time window, and then subsequently downregulated. This is followed by an increase in cell cycle related pathways over the 24h-48h time window, such as cell division and regulation of mitotic cell cycle phase transition. The upregulation of EC migration and angiogenesis during the late phase (72h-168h) suggests that after inflammatory injury and proliferation, repair of the lung endothelial barrier likely involves EC migration.

We also applied the Time-Heatmap analysis to brain ECs and heart ECs. We observed a similar juxtaposition of early host-defense responses followed by EC proliferation and regeneration (Figure 5—figure supplement 1 and 2). However, the regenerative responses in heart ECs showed a significantly delayed activation which was primarily seen at 168h, i.e., 1 week after the injury.

For mechanistic analyses, it is also critical to identify individual genes which drive the biological processes which is why we developed a function allowing for a visualization of fold-
change (logFC) expression of enriched gene sets from selected target GO terms. As shown in
Figure 6A, during the 0-6h time window the GO term defense response was enriched in brain,
heart and lung ECs, but in each vascular bed different sets of dynamic genes were involved. For
brain ECs (Figure 6A), the highly dynamic DDEGs were tumor necrosis factor (Tnf), aconitate
decarboxylase 1 (Acod1), interleukin 1 receptor (Il1r2), sialic acid binding Ig-like lectin E (Siglece),
chemokine ligand 1 (Cxcl1), interleukin 6 (Il6) and matrix metallopeptidase 12 (Mmp12); For heart
ECs (Figure 6B), the highly DDEGs are interleukin 18 receptor accessory protein (Il18rap), sialic
acid binding Ig-like lectin E (Siglece), interleukin 1 receptor (Il1r2), interleukin 6 (Il6), chemokine
ligand 5 (Cxcl5) and N-formyl peptide receptor 2 (Fpr2). Whereas, for lung ECs (Figure 6C), top
dynamic corresponding genes were triggering receptor expressed on myeloid cells 3 (Trem3),
arginase 1 (Arg1), interleukin 1 receptor type 2 (Il1r2), interleukin 10 (Il10), alpha 2-HS
glycoprotein (Ahsg). These organ-specific dynamic EC response genes may provide novel
vascular-bed novel therapeutic targets.

Organ-specific baseline differentially expressed genes (DEGs) temporal behavior
following endotoxemia

We next focused on the top organ-specific highly expressed differentially expressed genes
(DEGs) identified during homeostatic conditions (Jambusaria et al., 2020) to see whether these
organ-specific EC signature genes remain stable over time in the setting of inflammation or
whether they are also dynamic. For each organ, we focused on the study of the top 10 RiboTag
signature genes ranked by their log fold change (logFC) value compared to the other two organs
(Figure 7A, Supplementary file 5). We found the following selected genes which constitute the
organ-specific EC signatures during homeostasis are modulated during inflammatory injury. For
brain ECs, 7 out of 10 highly expressed brain EC signature genes were disrupted by the
inflammatory process (Figure 7A, Supplementary file 5), most of which showed rapid
downregulation. Only apolipoprotein D followed an “up-down” pattern, which was upregulated in the first 72 hours after inflammation and returned to baseline after 72 hours. For heart ECs (Figure 7B, Supplementary file 5), butyrophilin like 9 (Btnl9) and aquaporin-7 (Aqp7) followed a monotonic increase pattern and increased almost 2-fold at 168 hours after inflammatory activation. For lung ECs (Figure 7C, Supplementary file 5), only resistin like alpha (Retnla) was upregulated in the first 6 hours and then downregulated to baseline. In contrast to brain ECs, the top 10 signature genes for heart and lung ECs remained stable during the injury phase suggesting that brain ECs exhibit the greatest vulnerability to losing their homeostatic brain EC signature in the face of systemic LPS injury.
Discussion

ECs serve as a highly dynamic interface under normal physiological conditions and their organ-specific heterogeneity is essential for organ development, homeostasis, and tissue regeneration (Bautch, 2011). The control of tissue vasculature by ECs is vital for disease states ranging from inflammation to cancer (Augustin & Koh, 2017). ECs regulate inflammation by preventing coagulation, controlling blood flow, and trafficking proteins from blood to tissues (Pober & Sessa, 2007). RiboTag RNA-seq profiling enables in situ assessment of organ-specific endothelial translatomes (Cleuren et al., 2019), and by performing this analysis across time points, we were able to track how each organ’s endothelial translatomes changed over time in response to a stressor. Uncovering how organ-specific ECs respond to inflammation in a time-dependent manner may uncover new therapeutic targets and the corresponding temporal windows.

Using our computational platform, TrendCatcher, we identified time-dependent endothelial inflammatory response genes in different organs post endotoxemia, which could be broadly grouped into shared universal response genes across all three endothelial beds as well as response genes that were unique to specific endothelial beds. Approximately 85-95% of all organ-specific dynamic genes responded with biphasic trajectories, and most of the genes responding to LPS-injury reached their peak within 48 hours. We also observed significant universal upregulated inflammatory responses across all endothelial beds, which were host-defense-related pathways. The early host-defense-related dynamic inflammatory markers suggest an organ-universal self-defense mechanism, in which ECs from various vascular beds activate pro-inflammatory cytokines and likely facilitate the migration of immune cells to the infected sites.

We observed brain-specific EC downregulation of Wnt pathway genes during early inflammation. Emerging evidence shows crosstalk between oligodendrocytes and ECs, which is mediated by signaling through the Wingless and Int-1(WNT)/beta-catenin pathway (Manukjan,
Ahmed, Fulton, Blankesteijn, & Foulquier, 2020). Dysregulation of Wnt signaling results in a breakdown of blood brain barrier function (BBB) (Laksitorini et al., 2019; Martowicz et al., 2019), which is why our observation that Wnt pathway genes were predominantly downregulated in brain ECs is especially intriguing. It may indicate that Wnt downregulation mediates LPS-induced compromise of BBB function and constitutes a point of vulnerability for the brain endothelium. Since BBB integrity can be compromised by endotoxemia and is thought to exacerbate neurodegenerative diseases such as Alzheimer’s disease (Barton et al., 2019; L. M. Liu, Wan, Xia, Kalionis, & Li, 2014) the identification of Wnt pathway gene downregulation in our unbiased temporal analysis offers important new therapeutic targets for restoring BBB integrity in endotoxemia.

One of the key features of bacteremia and endotoxemia is the compromise of cardiac function (Kakihana, Ito, Nakahara, Yamaguchi, & Yasuda, 2016; G. Yucel et al., 2017). On the one hand, the myocardium responds with hyperdynamic function especially in the setting of systemic vasodilation to maintain systemic perfusion (Kalogeris, Baines, Krenz, & Korthuis, 2012), but prolonged endotoxemia or bacteremia can also lead to stress cardiomyopathy (Jafarzadeh, Thomas, Warren, Gill, & Fraser, 2016; Roivainen et al., 2000). As there is growing evidence that cardiac ECs are key regulators of cardiac function and that loss of cardiac contractility is in part due to a compromise of the cardiac endothelium (Potente & Mäkinen, 2017; N. Yucel et al., 2020), the gene expression changes we observed in cardiac ECs may reflect these distinct phases of endotoxemia or bacteremia. The early rise in ribosome biogenesis genes is followed by ER stress pathway upregulation, which suggests that ER stress in the cardiac endothelium may contribute to myocardial dysfunction during prolonged endotoxemia, and later, at 24 hours, heart EC activated genes were enriched in the endoplasmic reticulum (ER).

Lung ECs are of special interest during systemic inflammation and endotoxemia because lung vascular injury is a core pathogenic mechanism in the development of acute respiratory
distress syndrome (ARDS). Leukocytes have been shown to migrate to the lung microvasculature and interstitium during acute lung injury (ALI) (Claser et al., 2019; Senaldi, Vesin, Chang, Grau, & Piguet, 1994) and the upregulation of calcium signaling increases lung endothelial permeability (Tiruppathi, Minshall, Paria, Vogel, & Malik, 2002), which further exacerbates lung injury by leading to unfettered influx of immune cells and fluid into the lung alveolar space. Importantly, TrendCatcher identified upregulation of lung EC cell cycle pathways at 48h-72h, which coincides with what was recently reported for the kinetics of lung EC regeneration after endotoxemic injury (M. L. Liu et al., 2019).

In summary, we have developed a novel computational and visualization tool, TrendCatcher, to facilitate the interpretation of time-course RNA-seq data. TrendCatcher can identify dynamic differentially expressed genes (DDEGs), assign dynamic trajectory patterns to DDEGs, reveal dynamic pattern composition across different experiments, and visualize changes in biological pathway enrichment across multiple time-points. Compared to existing computational tools, it achieved higher prediction power in dynamic gene identification from simulated datasets. Since TrendCatcher assesses distinct time points sequentially and thus maintains a temporal hierarchy, it may be better suited for the identification of complex temporal trajectories than current methods. By allowing users to set fold-change thresholds as well as define a priori gene sets of interest, TrendCatcher is versatile and can be used to mine a variety of temporal RNA-seq datasets. By applying it to EC transcriptomics, we identified injury and regeneration kinetics across various vascular-beds which could pave the way for assessing novel therapeutic targets during defined temporal windows.
Materials and Methods

TrendCatcher framework

The main components of the TrendCatcher framework are shown in Figure 1. TrendCatcher requires two main inputs: the raw count table \( C \) of a temporal study with a dimension of \( m \times n \), where \( m \) denotes the number of genes and \( n \) denotes the number of samples, and a user-defined baseline time vector \( T \), such as “0 hour”. Since samples may have different sequencing depths and batch effect, TrendCatcher integrates with limma (Ritchie et al., 2015) and provides preprocessing steps. Based on a user-specified threshold, relatively low abundant genes are removed from the count table, and reads are normalized and batch effects are removed. TrendCatcher’s core algorithm is composed of five main steps: (a) baseline fluctuation confidence interval estimation, (b) model dynamic longitudinal count, (c) time point dynamic p-value calculation, (d) gene-wise dynamic p-value calculation, and (e) break point screening and gene-wise dynamic pattern assignment. Mathematical details will be expanded in the following sections.

For the output of TrendCatcher, there are mainly two components: a master table and a set of functions for versatile visualization purposes. The master table contains all the dynamic details of each single gene, including its dynamic p-value, its break point location time, and its dynamic trajectory pattern. In addition to the master table, TrendCatcher produces three main types of visualizations: (a) a figure showing the observed counts and fitted splines of each gene, (b) a hierarchical pie chart that represents trajectory pattern composition, (c) and a Time-Heatmap of dynamic biological pathway enrichment change over time.

Baseline fluctuation confidence interval estimation

We assumed that the observed number of RNA-seq reads count from the baseline time (e.g., \( t=0 \) hour) \( X_{i,t_{baseline}} \) was generated from a negative binomial distribution (Equation 1):
Where gene $i = 1, \ldots, n$ and $\mu_{i,t_{\text{baseline}}}$ is the mean count of gene $i$ at baseline time, and $\varphi_i$ is the dispersion factor. First, the dispersion factor $\varphi_i$ was pre-estimated as a constant hyperparameter for each gene with DESeq2 (Love et al., 2014), as shown in Equation 2. Here, $\sigma_i$ is the variance.

$$\sigma_i(t)^2 = \mu_i(t) + \varphi_i \cdot \mu_i(t)^2$$

Then, $\mu_{i,t_{\text{baseline}}}$ was estimated using maximum likelihood from Equation 3.

$$\hat{\mu}_{i,t_{\text{baseline}}} = \arg \max_{\mu, \varphi} L_{NB}(u, \varphi) = \arg \max \frac{\Gamma(\varphi + x)}{x! \Gamma(\varphi)} \left( \frac{\mu}{\mu + \varphi} \right)^x \left( \frac{\varphi}{\varphi + \mu} \right)^\varphi$$

Based on negative binomial distribution and the estimated mean count for baseline time, we constructed the 95% confidence interval $[X_{i,t_{\text{baseline}}, 0.05}, X_{i,t_{\text{baseline}}, 0.95}]$ as the baseline count fluctuation interval (Equation 4(a) and Equation 4(b)).

$$P(X_{i,t_{\text{baseline}}} \leq X_{i,t_{\text{baseline}}, 0.05} | \mu_{i,t_{\text{baseline}}}, \varphi_i) = 0.05$$

$$P(X_{i,t_{\text{baseline}}} \geq X_{i,t_{\text{baseline}}, 0.95} | \mu_{i,t_{\text{baseline}}}, \varphi_i) = 0.95$$

**Model dynamic longitudinal count estimation**

To model the time dependent gene expression value, we applied a smoothing spline ANOVA model (Gu, 2013; Metwally et al., 2018) with a negative binomial family constrain to fits the reads from samples across non-baseline multiple time points. The random variable $X_{i,t(t \neq t_{\text{baseline}})}$ is assumed to follow NB distribution in Equation 5, with a positive integer $\alpha$ represents number of failures before the $\alpha$th success in a sequential of Bernoulli trials, and $p(t) \in (0, 1)$ represents the success probability.

$$X_{i,t(t \neq t_{\text{baseline}})} \sim NB(\alpha, p(t))$$

The log likelihood given a time-course observed count $x = \{x_{i,t(t \neq t_{\text{baseline}})}\}_{i=1,...,n; t=1,...,T}$ is calculated as Equation 6.
\[ L = \log L(\alpha, p(t) \mid X = x) \]
\[ = \sum_{t=1}^{T} \sum_{i=1}^{n} \left[ x_{i,t(t \neq \text{baseline})} \log(1 - p(t)) + a \log p(t) + \log \Gamma(\alpha + x_{i,t(t \neq \text{baseline})}) \right] - \log \log \Gamma(\alpha) + C \]  

(6)

415 Taking the logit link and model time effect, we define the logit link \( \eta \)

\[ \eta = \log \frac{p(t)}{1 - p(t)} \]  

(7)

416 To allow for flexibility in the estimation of \( \eta \), and find the best trade-off between goodness of fit and the smoothness of the spline curve, soft constraints of the form \( J(\eta) \) is added to the minus log-likelihood, with the smoothing parameter \( \lambda > 0 \).

\[ -L + \lambda J(\eta) \]

(8)

419 The solution to the optimization of Equation 8 leads to a smoothing fitting to the reads from samples across different non-baseline time points. The estimated mean of \( \mu_{i,t(t \neq \text{baseline})} \) can be estimated using Equation 9.

\[ \mu_{i,t(t \neq \text{baseline})} = \hat{\alpha} e^{\hat{\eta}(t)} = \frac{\hat{\alpha} \hat{p}(t)}{1 - \hat{p}(t)} \]  

(9)

423 Gene’s dynamic p-value calculation

424 To calculate gene’s non-baseline dynamic signal significance, each gene’s non-baseline estimated mean count \( \mu_{i,t(t \neq \text{baseline})} \) was tested against the baseline fluctuation interval. Based on Equation 10(a) and Equation 10(b), for each gene at each single non-baseline time point, a dynamic time p-value was calculated.

\[ \text{If } \mu_{i,t(t \neq \text{baseline})} \geq \mu_{i,t \text{baseline}} \text{ then } p_{i,t} = \int P(x \geq \mu_{i,t(t \neq \text{baseline})} \mid \mu_{i,t \text{baseline}}, \varphi_i) \]  

(10a)

\[ \text{If } \mu_{i,t(t \neq \text{baseline})} \leq \mu_{i,t \text{baseline}} \text{ then } p_{i,t} = \int P(x \leq \mu_{i,t(t \neq \text{baseline})} \mid \mu_{i,t \text{baseline}}, \varphi_i) \]  

(10b)
Then, we applied Fisher’s combined probability test method to calculate a gene-wise dynamic p-value (Equation 11):

\[ \chi^2_{i,2t} \sim -2 \sum_{t=0}^{T} \ln(p_{i,t}) \]

(11)

**Trajectory pattern assignment**

First, we connect all the significant dynamic signal time points, with a p-value threshold less than 0.05. Then we applied a break point searching strategy to capture the gene expression change trend. The definition of break point is defined using Equation 12(a) and Equation 12(b).

\[
\begin{align*}
\text{If } &\mu_{i,t} > \mu_{i,t_{next}} \text{ and } \mu_{i,t} > \mu_{i,t_{previous}}, \text{break point type I.} \\
\text{If } &\mu_{i,t} < \mu_{i,t_{next}} \text{ and } \mu_{i,t} < \mu_{i,t_{previous}}, \text{break point type II.}
\end{align*}
\]

(12a)

There are two types of break points, type I means gene up-regulated followed by a down regulation and type II means gene expression level down down regulated and then followed by an upregulation. By screening along the break point, the master-pattern and sub-pattern were assigned to each gene.

**Time-Heatmap enrichment analysis**

To build the Time-Heatmap for visualizing the biological pathway enrichment change over time, we designed a window-sliding strategy to capture all the up-regulated or down-regulated genes for each time interval. If we denote time vector as \(t_j\) and \(j \in 1, ..., T\), each time interval is denoted as \([t_{j-1}, t_j]\). We found \(N_{up}\) up-regulated genes and \(N_{down}\) down-regulated genes within the time window \([t_{j-1}, t_j]\), then a Fisher’s exact test was performed to obtain the Gene Ontology (GO) term enrichment with the corresponding time interval.
Besides GO enrichment analysis (Yu, Wang, Han, & He, 2012), TrendCatcher also packed Enrichr (Kuleshov et al., 2016) biological pathway databases.

Simulated dataset

We embedded 5 different types of trajectories into the temporal RNA-seq simulated datasets, including constant trajectory, linear trajectory, impulse trajectory, 2 break-point trajectory and 3 break-point trajectory. Each type of trajectory was constructed by adding negative binomial distribution noise to the embedded trajectory count. We mixed 1,000 constant trajectories with the other 4 types of dynamic trajectories (500 from each type). Then, we used a dynamic filter to trajectories to further distinguish constant trajectories versus strong dynamic trajectories. We used log2FC range over the time course less than 0.5 as a threshold to relabel constant trajectories and strong dynamic trajectories. We sampled 6 to 10 different number of time points with evenly time intervals, and randomly sampled 3 replicates for each time point.

RiboTag dataset preprocessing and batch correction

We downloaded RiboTag RNA-seq dataset from NCBI-GEO website with the access number GSE136848. We applied limma R package for library normalization and batch correction to the Brain EC, Heart EC and Lung EC respectively. For gene symbol annotation, we used ENSEMBL features from biomaRt (Durinck, Spellman, Birney, & Huber, 2009) package.
Acknowledgments

The studies were supported by NIH grants R01-HL152515 (to JR), R01-HL154538 (to JR), P01-HL060678 (to JR) and R01-HL126516 (to JR).
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Figure 1. TrendCatcher Framework
Figure 2. TrendCatcher prediction performance. (A) Prediction accuracy compared to DESeq2, DESeq2Spline and ImpulseDE2 model with mixed types of trajectory patterns. (B) Prediction recall from the same simulated dataset with (A). (C) Prediction precision from the same simulated dataset with (A). (D) Prediction accuracy compared to DESeq2, DESeq2Spline and ImpulseDE2 model with various number of time points, from 6 to 10, using mixed types of trajectory patterns.
Figure 3. Dynamic gene composition comparison among brain, heart and lung ECs. (A) Number of different types of dynamic gene trajectories belonged to each master-pattern. (B) Hierarchical pie chart of brain EC, inner pie is master-pattern composition, outer pie is sub-pattern composition. (C) Hierarchical pie chart of heart EC. (D) Hierarchical pie chart of lung EC.
Figure 4. Organ-universal inflammatory response, and organ-specific inflammatory response for trajectory sub-pattern, "0h_up_6h_down_168h". (A) Venn diagram of dynamic genes from sub-pattern "0h_up_6h_down_18h" across brain, heart and lung ECs. (B) GO enrichment for the overlapped 143 dynamic genes. X-axis is the number of genes, y-axis is GO term, p-value is the enrichment p-value for each GO term. (C) GO enrichment analysis for 106 genes only found in brain EC. (D) GO enrichment analysis for 629 genes only found in heart EC. (E) GO enrichment analysis for 702 genes only found in lung EC.
Figure 5. Time-heatmap of lung ECs. Each column represents a time window, each row represents a significantly enriched (p-value <0.05) GO term. Red shade represents GO term are upregulated compared to the previous break point. Blue shade represents GO term are down regulated compared to the previous break point. Percentage number within each tile represents the percentage of genes belong to the corresponding GO term are dynamic and captured by TrendCatcher. Shade of the color represents the enrichment p-value, the darker the shade is, the more significantly enriched from GO enrichment analysis.
Figure 6. GO-Heatmap. For time window 0h-6h, from selected pathways, including "regulation of defense response", "leukocyte migration", "myeloid leukocyte migration", "leukocyte chemotaxis", "granulocyte chemotaxis", "cellular response to chemokine", "chemokine-mediated signaling pathway", "angiogenesis", "sprouting angiogenesis", "response to bacterium", "leukocyte mediated immunity". Dynamic genes from each organ. (A) Brain EC. (B) Heart EC. (C) Lung EC. The color shade represent log fold change magnitude. Red means up-regulated, blue means down-regulated.
Figure 7. Top 10 organ-specific RiboTag EC signature genes dynamic trajectories. (A) Brain ECs top 10 signature genes trajectories, with gene symbol, dynamic p-value from TrendCatcher and its trajectory pattern. (B) Heart ECs top 10 signature genes trajectories. (C) Lung ECs top 10 signature genes trajectories. 

dyn.p.adj is the adjusted dynamic p-value from TrendCatcher. Black dots are observed count data for each time point for each gene. Red line is smoothed ANOVA spline fitting. Grey lines are baseline fluctuation intervals constructed using constant NB model.
Figure 2-figure supplement 1. TrendCatcher prediction performance across different types of trajectories. (A) Prediction accuracy (left), recall (top-right) and precision (bottom-right), compared to DESeq2, DESeq2Spline and ImpulseDE2 model with linear trajectories. (B) Prediction accuracy (left), recall (top-right) and precision (bottom-right), compared to DESeq2, DESeq2Spline and ImpulseDE2 model with impulse trajectories. (C) Prediction accuracy (left), recall (top-right) and precision (bottom-right), compared to DESeq2, DESeq2Spline and ImpulseDE2 model with two break points trajectories. (D) Prediction accuracy (left), recall (top-right) and precision (bottom-right), compared to DESeq2, DESeq2Spline and ImpulseDE2 model with three break points trajectories.
Figure 3–figure supplement 1. Top sub-pattern dynamic gene trajectories in lung ECs. Ranked by the number of sub-pattern trajectory type. "0h up 48h down" means gene upregulated in the first 48 hours and then downregulated from 48 hours to 168 hours. X-axis is time (hour), y-axis is the log2 count of RNA-seq reads.
Figure 3–figure supplement 2. Top sub-pattern dynamic gene trajectories in brain ECs. Ranked by the number of sub-pattern trajectory type. X-axis is time (hour), y-axis is the log2 count of RNA-seq reads.
Figure 3–figure supplement 3. Top sub-pattern dynamic gene trajectories in heart ECs. Ranked by the number of sub-pattern trajectory type. X-axis is time (hour), y-axis is the log2 count of RNA-seq reads.
Figure 4–figure supplement 1. Organ-universal inflammatory response, and organ-specific inflammatory response for trajectory sub-pattern, "0h_down_6h_up_168h". (A) Venn diagram of dynamic genes from sub-pattern "0h_down_6h_up_168h" across brain, heart and lung ECs. (B) GO enrichment analysis for 90 genes only found in brain EC. (C) GO enrichment analysis for 101 genes only found in heart EC. (D) GO enrichment analysis for 543 genes only found in lung EC.
**Figure 4—figure supplement 2.** Organ-universal inflammatory response, and organ-specific inflammatory response for trajectory sub-pattern, "0h_up_24h_down_168h". (A) Venn diagram of dynamic genes from sub-pattern "0h_up_24h_down_168h" across brain, heart and lung ECs. (B) GO enrichment analysis for 307 genes only found in brain EC. (C) GO enrichment analysis for 332 genes only found in heart EC. (D) GO enrichment analysis for 110 genes only found in lung EC.
**Figure 5—figure supplement 1.** Time-heatmap of brain ECs. Each column represents a time window, each row represents a significantly enriched (p-value <0.05) GO term. Red shade represents GO term are upregulated compared to the previous break point. Blue shade represents GO term are down regulated compared to the previous break point. Percentage number within each tile represents the percentage of genes belongs to the corresponding GO term are dynamic and captured by TrendCatcher. Shade of the color represents the enrichment p-value, the darker the shade is, the more significantly enriched from GO enrichment analysis.
Figure 5—figure supplement 2. Time-heatmap of heart ECs. Each column represents a time window, each row represents a significantly enriched (p-value < 0.05) GO term. Red shade represents GO term are upregulated compared to the previous break point. Blue shade represents GO term are down regulated compared to the previous break point. Percentage number within each tile represents the percentage of genes belongs to the corresponding GO term are dynamic and captured by TrendCatcher. Shade of the color represents the enrichment p-value, the darker the shade is, the more significantly enriched from GO enrichment analysis.