Effects of destrin pathway mutations on the gene expression profile


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ABSTRACT: This study aimed to explore the interaction and crosstalk between pathways in response to destrin mutations. All the pathways from the MINT database were downloaded, a protein-protein interaction network was then constructed, and the crosstalk between pathways was investigated, in particular, the overlap of 2 significant pathway analysis results. As expected, the results showed that regulation of the actin cytoskeleton was the significant pathway of destrin mutations in mice. Further analysis indicated that 28 significant pathways cross-talked with the pathway regulating the actin cytoskeleton. Importantly, 3 pathways, including regulation of actin cytoskeleton pathway, pathways in cancer, and the B cell receptor signaling pathway were linked by inositol phosphate metabolism based on crosstalk analysis of Gene Ontology relationships among pathways. All of these pathways have been demonstrated to participate in cytoskeleton dynamics. These findings might provide valuable insights into cytoskeleton dynamic abnormalities in destrin mutations of corneal diseases.

Key words: ADF/Cofilin; Cytoskeleton dynamics; Pathway crosstalk
INTRODUCTION

Corneal disease is responsible for 6% of legal blindness in the United States and is frequently the main cause of bilateral blindness in the world, second only to cataract (Ikeda et al., 2003; Li et al., 2009). Visual loss in many corneal diseases is due to changes in the morphology and function of the corneal epithelial surface, including cell hyperproliferation, inflammation, and angiogenesis (Dawson et al., 2009).

Destrin (also known as actin-depolymerization factor, ADF) is an essential actin regulatory protein of the ADF/cofilin family that binds to the actin subunits of filamentous actin (F-actin), enhancing the subunit off-rate and promoting filament severing. Thus, this family of proteins is responsible for increasing the turnover of actin filaments and is involved in the regulation of cytoskeleton dynamics (Tokuraku et al., 2001; Maciver and Hussey, 2002). Dstn\textsubscript{corn1} mice represent a spontaneous mutant line that exhibits ocular surface abnormalities shortly after birth and therefore often serves as a good model to study corneal disease. The histology of Dstn\textsubscript{corn1} corneas showed that the hyperplastic corneal epithelium expressed an increased level of keratin 14 and involucrin, while the level of keratin 12 was not altered (Zhang et al., 2008). The normal cornea is deficient in blood and lymphatic vessels to maintain corneal transparency, unless severe inflammatory or other strains cause a disruption of the antiangiogenic privilege of the cornea. It has been shown that hemangiogenesis and lymphangiogenesis in the Dstn\textsubscript{corn1} cornea depend on vascular endothelial growth factor receptor 3 (VEGFR3) signaling (Cursiefen et al., 2005). Recent reports have suggested that vascularization of Dstn\textsubscript{corn1} corneas arises from the lack of soluble VEGFR, sflt-1, which has been proposed as an essential factor for maintenance of avascularity in a normal cornea. Suppression of endogenous sflt-1 by neutralizing antibodies, RNA interference, or Cre-lox-mediated gene disruption abolishes corneal avascularity in mice, but recombinant sflt-1 administration restores corneal avascularity in corn1 and Pax6+/- mice (Ambati et al., 2006).

Genome-wide screening of differentially expressed genes (DEGs) in the cornea of Dstn\textsubscript{corn1} mice reveals that the expression of a large portion of genes associated with cytoskeletal dynamics was upregulated (Verdoni et al., 2008). Nearly half of these genes are targets of the serum response factor (SRF), an essential regulator of the actin cytoskeleton (Miano et al., 2007; Miano, 2008). The conditional ablation of Srf in the corneal epithelium of a diseased Dstn\textsubscript{corn1} cornea results in the rescue of epithelial cell hyper-proliferation, inflammation, and neovascularization phenotypes (Verdoni et al., 2010). These results indicate that there is an underlying interaction among these DEGs. Given the complex nature of biological systems, pathways often need to function in a coordinated fashion to produce appropriate physiological responses to both internal and external stimuli (Li et al., 2008). Therefore, we performed research on the protein-protein interaction (PPI), significant pathway, and crosstalk between pathways based on the previous study in our institution, with the hope to lay an important theoretical foundation for understanding the molecular mechanism of destrin in corneal diseases.

MATERIAL AND METHODS

Data sources

We download all the pathways from KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa, 2002) and all the PPI datasets from MINT (the Molecular Interaction da-
tabase) (Ceol et al., 2010), which contains the mouse PPI datasets from IntAct (Aranda et al., 2010), BIOGRID (the Biological General Repository for Interaction Datasets) (Stark et al., 2011), and HPRD (Human Protein Reference Database) (Keshava Prasad et al., 2009).

Next, an ensemble PPI network was constructed by integrating 2 of the above-described PPI databases in mice. A total of 65,851 unique PPI pairs were collected, involving 10,951 unique proteins.

We extracted the gene expression profile data for the Dstn mutations with normal wild-type from Verdoni et al. (2008), which were deposited in NCBI (National Center for Biotechnology Information) GEO (Gene Expression Omnibus database, http://www.ncbi.nlm.nih.gov/geo/) database (ID: GSE9743). All mouse procedures were performed in accordance with the protocols approved by the Animal Care and Use Committee at the University of Wisconsin-Madison and conformed to the ARVO statement for the use of animals in Ophthalmic and Vision Research and APS’s Guiding Principals in the Care and Use of Animals. The Dstn mutations and wild-type samples with 6 replications in each group were compared.

The Limma package in R language (Smyth, 2004) was used to identify DEGs. Background intensities were adjusted, and the original expression datasets from all conditions were processed into expression estimates using the RMA method with the default settings implemented in R (version 2.12.1) to construct the linear model. Only the DEGs with fold-change values greater than 1.5 and P values less than 0.05 were selected.

Pathway crosstalk analysis

The crosstalk pathways are defined as those pathways that have overlapping genes and edges. The overlapping genes mean that both pathways are included, and the overlapping edges mean that both pathways included the PPI interaction edges.

To determine the co-expressed significance of a gene pair in disease cases, we used the Pearson correlation coefficient test to calculate the P value.

The P values were mapped to the nodes and edges of the PPI network collected from MINT. The following formula was used to define a function as the combination of the statistical significance of an interaction according to a scoring matrix. The detailed description can be found in Liu et al. (2010).

\[
S(e) = f(diff(x), cor(x, y), diff(y))
\]

\[
= 2 \sum_{i=1}^{k} \log(p_i)
\]

Functions \(diff(x)\) and \(diff(y)\) are differential expression assessments of gene \(x\) and gene \(y\), respectively. Function \(cor(x,y)\) represents the correlation between gene \(x\) and gene \(y\). Method \(f\) is a general data integration method that can handle multiple data sources differing in statistical power. When \(k = 3\), \(p1\) and \(p2\) are the P values of differential expression of 2 nodes, and \(p3\) is the P value of their co-expression.

Significant pathways analysis

\[
Sp = \sum_{e \in P} S(e)
\]
The scores that are larger than Sp are used as the significance P value of pathway P to describe its importance. We also used DAVID (Huang et al., 2009) for pathway-enrichment analysis for P values <0.05 as input into the DEG dataset.

**Crosstalk analysis of relationships among pathways**

The detailed crosstalk analysis of relationships among pathways was then investigated, especially that of overlap between 2 significant pathway analysis results. To define the interaction significance between pathways, we summarize all the scores of edges S(e) of all non-empty overlaps. Specifically, the interaction score between 2 pathways was estimated by their overlapping status of weighted pathways using the following formula:

\[ C(pi, pj) = \sum_{e \in O_{ij}} S(e), \]

where \( P_i \) and \( P_j \) are 2 pathways and \( O_{ij} \) is their overlap.

To estimate the significance of the overlap between different pathways, we randomly sampled \( 10^5 \) times the same size of the 2 pathways from the edges of the pathway network and calculated their overlap scores. The frequency larger than \( C \) is regarded as the interaction significance P values. Finally, the crosstalk with P values <0.001 were considered to be significant.

**Significant Gene Ontology (GO)-enrichment analysis of each pathway**

The functional enrichment among proteins in 1 pathway is defined as follows:

\[ P = 1 - \sum_{i=0}^{k-1} \frac{\binom{f}{i} \binom{n-f}{m-i}}{\binom{n}{m}}, \]

where \( n \) is the number of nodes in the network, \( f \) is the number of proteins annotated with a particular GO function, \( m \) is the number of proteins involved in the pathway, and \( k \) is the frequency of the GO term. We identified the GO function enrichment of the pathways.

**RESULTS**

In this study, the GSE9743 dataset was first downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/), and the R language was then used to calculate the DEGs. Based on the expression profiles, we utilized the PPI dataset and the KEGG pathways to elucidate significant pathways and the crosstalk among these significant pathways.

**Significant pathway analysis**

We used Sp to evaluate the importance of pathways (for details, see Material and Methods section). Thirty-six pathways (Table 1) were detected with P values <0.01.
The Limma package was used to detect 120 DEGs (for details, see Material and Methods section). Using DAVID with the DEGs, several pathways were identified. However, we only found 4 significant pathways: regulation of actin cytoskeleton (mmu04810) with a P value = 0.01, leukocyte transendothelial migration (mmu04670) with a P value = 0.001, tight junction (mmu04530) with a P value = 0.017, and arrhythmogenic right ventricular cardiomyopathy (mmu05412) with a P value = 0.04.

Remarkably, only 1 overlap of a significant pathway (regulation of actin cytoskeleton, mmu04810, marked red in Table 1), was detected in the Dstn mutations.

Crosstalk among the pathways

Further, we exploited the pathway crosstalk between regulation of actin cytoskeleton (mmu04810) and other significant pathways using the overlapping score. We found that 28 significant pathways cross-talked to the mmu04810 pathway (Table 2).

<table>
<thead>
<tr>
<th>ID</th>
<th>Node</th>
<th>Edge</th>
<th>Size</th>
<th>Score</th>
<th>P value</th>
<th>Description</th>
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<td>218</td>
<td>3823.94</td>
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<td>0</td>
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<td>101</td>
<td>161.86</td>
<td>0.00877</td>
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</tr>
</tbody>
</table>

Table 1. Significant pathway analysis.
Effects of destrin mutations on gene expression

For detailed analysis of the crosstalk between the significant pathways, we used the hypergeometric test to identify the significant GO terms of each pathway, with P values <0.05. The results of the top 5 GO terms in parts of the pathways were used to construct the connection among the pathways. In Figure 1, regulation of actin cytoskeleton (mmu04810) connected with inositol phosphate metabolism (mmu00562) through cell adhesion (GO: 007155), with a crosstalk P value <0.01.

Inositol phosphate metabolism (mmu00562), pathways in cancer (mmu05200), and B cell receptor signaling pathway (mmu04662) were connected through signal transduction (GO: 007165).

Based on the significant GO enrichments, we predicted the crosstalk between the GO biological processes during the disease development among the pathways.
DISCUSSION

Dstn<sup>com1</sup> mice exhibit an actin dynamic defect in corneal epithelial cells, offering an in vivo model to investigate cellular mechanisms affected by the Dstn mutation and resultant actin dynamic abnormalities. Microarray analysis using the cornea from Dstn<sup>com1</sup> and wild-type mice demonstrated that Dstn mutations have a strong influence on the gene expression profile, especially on the actin cytoskeleton regulator. As anticipated, regulation of the actin cytoskeleton (mmu04810) was also a significant pathway in our analysis. Furthermore, 28 significant pathways cross-talked to the mmu04810 pathway and were identified using the overlapping score (Table 2). Importantly, crosstalk analysis of GO relationships among pathways indicated that the mmu04810 pathway was indirectly connected to the mmu05200 pathway (pathways in cancer) and the mmu04662 pathway (B cell receptor signaling pathway) in a mmu00562...
pathway-mediated manner (inositol phosphate metabolism). The results indicate that there are interaction relationships among these 4 pathways, which is in accordance with previous reports as follows.

Recent studies demonstrated that the organization and dynamics of the actin cytoskeleton could be regulated by the phosphoinositide pathway at several levels, such as phosphatidylinositol-3,4,5-trisphosphate (PIP3), phosphatidylinositol-4,5-bisphosphate (PIP2), and the enzymes catalyzing the production or hydrolysis of these lipids. Therefore, crosstalk between regulation of actin cytoskeleton and the phosphoinositide pathway is expected. Among the different PIs, PI(4,5)P2 is the best-characterized regulator of the actin cytoskeleton. PI(4,5)P2 interacts directly with several actin-binding proteins, such as ADF/cofilin, to regulate the activities of the actin-binding proteins (Zhao et al., 2010). Typically, PI(4,5)P2 inhibits actin-binding proteins that promote actin filament disassembly and activates proteins that induce actin filament assembly (van Rheenen et al., 2007). Therefore, PI(4,5)P2 is considered a promoter of the formation of actin filament structures beneath the plasma membrane and other phosphoinositide-rich membrane organelles. This is supported by a number of studies demonstrating that an increased plasma membrane PI(4,5)P2 level induces actin filament assembly in mammalian cells, while sequestration of PI(4,5)P2 leads to a defective cortical actin cytoskeleton (Saarikangas et al., 2010).

One study has demonstrated that there is a relationship between the regulation of the actin cytoskeleton and B cell receptor (BCR) signaling in the antigen processing and presentation process. Bruton’s tyrosine kinase (Btk) is one linker connecting BCR signaling to actin dynamics. Using xid mice and a Btk inhibitor, BCR engagement increases actin polymerization and Wiskott-Aldrich syndrome protein (another actin-binding protein, similar to ADF/cofilin) activation in a Btk-dependent manner. Concurrently, Btk-dependent increases based on the level of PIP2, and phosphorylated Vav is observed upon BCR engagement. Thus, the BCR-triggered signaling regulates the dynamics of the actin cytoskeleton through WASP in a Btk-dependent manner (Sharma et al., 2009).

Cell hyperproliferation, inflammation, and angiogenesis are biological processes central to the pathogenesis of corneal disease, as well as other conditions including tumorigenesis and chronic inflammatory disorders. Therefore, pathways in cancer may be involved in the regulation of the actin cytoskeleton. In the past decade, many signaling pathways have been identified to be associated with cancer development, such as Ras/MAPK, MAPK/ERK, TGF-β, and PI3K (Dreesen and Brivanlou, 2007). These pathways have all been proposed to influence the regulation of the actin cytoskeleton. For example, the Ras/MAPK pathway is likely the critical pathway involved in cytoskeleton disruption during Ras transformation. Oncogenic Ras can specifically target the actin-based cytoskeleton and achieve morphological transformation of the cells by down-regulation of structural components of the cytoskeleton and inhibition of ROCKI/Rho kinase-dependent pathways (Pawlak and Helfman, 2002; Samaj et al., 2004). TGF-β, via Smad and p38Mapk, up-regulates expression of actin-binding proteins such as ADF/cofilin to regulate the actin cytoskeleton and cell motility in epithelial cells (Bakin et al., 2004; Vardouli et al., 2005; Moustakas and Heldin, 2008). One study demonstrated that the JNK and PI3K signaling cascades initiate in the early stages of angiogenesis through the reorganization of the actin cytoskeleton to increase production and activation of MMP-2. However, JNK mainly regulates the mRNA expression of MMP-2 and MT1-MMP, whereas PI3K regulates protein levels (Ispanovic and Haas, 2006).
In conclusion, in this paper, a network-based approach was used to analyze the cross-talk between corneal related pathways. The crosstalk between the pathways was identified and analyzed using PPI datasets and expression profiles. The results are consistent with prior knowledge of actin dynamics. The crosstalk of pathways presents new alternative insights for corneal disease. As a comprehensive and system-wide analysis, our research may provide evidence for corneal disease and complements traditional component-based approaches.

REFERENCES


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RETRACTION