Examining the Pathophysiology of Lichen Sclerosus: Comparison of miRNA Expression Profiles between Lichen Sclerosus and Non-Lichen Sclerosis Induced Urethral Strictures
I have no financial disclosure or conflict of interest with the presented material in this presentation.
To examine the pathophysiology of Lichen Sclerosus (LS) and non-LS urethral stricture disease (USD) by comparing microRNA expression profiles in tissue samples from men undergoing urethroplasty
Background
What is Lichen Sclerosus?

- Chronic inflammatory condition which is presumed to be the underlying etiology of approximately 14-29% of cases of male USD

- Characterized by scarring of the urethra which varies in length and location, occurring anywhere from the urethral meatus to the membranous urethra
Background

Treatment for LS USD

- Urethroplasty is the gold standard

- Overall USD urethroplasty success rates range from 84-92%

- Urethroplasty success rates in patients with LS USD is significantly worse due to high recurrence rates
Pathophysiology of LS USD

- Pathophysiology of USD and specifically LS USD is poorly understood
- No studies have evaluated miRNA expression in LS USD
Background

What are microRNA?

- MicroRNA (miRNA) are non-coding genetic material involved in the regulation of gene expression
- Have shown potential to differentiate behaviors of cancer as well as be involved in regulation of fibrosis
Materials and Methods

- LS and non-LS urethral stricture tissue samples collected between 2005-2017

- Pathologic evaluation of strictures were based on histologic features considered diagnostic of LS
Materials and Methods

● 5 typical histologic features of LS:
  (1) hyperkeratosis
  (2) thinning or thickening of the squamous epithelium
  (3) attenuation or vacuolar degeneration of the basal cell layer
  (4) subepithelial hyalinization
  (5) lichenoid lymphoplasmacytic infiltrate

● 3-5 features: diagnostic of LS
  2 features: suggestive of LS
  0-1 features: negative for LS
Total RNA was extracted from 13 LS urethral strictures and 13 non-LS urethral strictures.

Samples were profiled via miRNA RT-qPCR arrays for 752 unique miRNA.

Gene Ontology analysis performed to predict biological processes involving identified miRNA.
Results

Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=26</th>
<th>LS (n=13)</th>
<th>Non-LS (n=13)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>0.511</td>
</tr>
<tr>
<td>range</td>
<td>34-80</td>
<td>49-73</td>
<td>34-80</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (median)</td>
<td>30.9 (30.8)</td>
<td>31.6 (32.9)</td>
<td>27.3 (28.9)</td>
<td>0.101</td>
</tr>
<tr>
<td>Smoking History (%)</td>
<td>11 (42.3)</td>
<td>6 (46.2)</td>
<td>5 (38.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>6 (23.1)</td>
<td>4 (30.8)</td>
<td>2 (15.4)</td>
<td>0.645</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>14 (53.8)</td>
<td>8 (61.5)</td>
<td>6 (46.2)</td>
<td>0.695</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>9 (34.6)</td>
<td>6 (46.2)</td>
<td>3 (23.1)</td>
<td>0.411</td>
</tr>
</tbody>
</table>

There were no significant differences regarding patient age, BMI, smoking history, or medical comorbidities such as hypertension, diabetes, and hyperlipidemia between the LS vs non-LS groups.
Each of 13 LS cases included in this study received a histopathologic score of 5.

13 non-LS cases had scores ranging from 0-1: nine patients with a score of 0 and four patients with a score of 1.
Results

miRNA Expression

- 752 miRNA analyzed, a total of 143 miRNA were detected for all samples
- 27 miRNA were found to be differentially expressed between the LS and non-LS groups (FDR <0.01)
Results

miRNA Expression

- 7 were found to be upregulated in LS with fold change >2, and 6 were found to be downregulated in LS with fold change <-2

- 15 of these 27 miRNA each achieved an area under the curve (AUC)>0.90 for discriminating between LS and non-LS strictures
### Results

**miRNA Expression**

<table>
<thead>
<tr>
<th>microRNA</th>
<th>t-test p-value</th>
<th>FDR q-value</th>
<th>AUC</th>
<th>Median FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-155-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>11.3</td>
</tr>
<tr>
<td>hsa-miR-146a-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.988</td>
<td>7.7</td>
</tr>
<tr>
<td>hsa-miR-150-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>6.2</td>
</tr>
<tr>
<td>hsa-miR-342-3p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.953</td>
<td>2.2</td>
</tr>
<tr>
<td>hsa-miR-142-3p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.917</td>
<td>4.0</td>
</tr>
<tr>
<td>hsa-miR-142-5p</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.876</td>
<td>2.4</td>
</tr>
<tr>
<td>hsa-miR-146b-5p</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.870</td>
<td>2.9</td>
</tr>
</tbody>
</table>

- miR-155-5p specifically was found to be upregulated by 11 fold in LS vs. non-LS strictures (p<0.001, AUC=1.0)
### Results

**miRNA Expression**

<table>
<thead>
<tr>
<th>microRNA</th>
<th>t-test p-value</th>
<th>FDR q-value</th>
<th>AUC LS vs NLS</th>
<th>Median FC LS vs NLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-99a-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.964</td>
<td>-2.7</td>
</tr>
<tr>
<td>hsa-miR-125b-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.947</td>
<td>-2.2</td>
</tr>
<tr>
<td>hsa-miR-424-5p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.905</td>
<td>-2.6</td>
</tr>
<tr>
<td>hsa-miR-376a-3p</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.923</td>
<td>-2.4</td>
</tr>
<tr>
<td>hsa-miR-30a-5p</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.882</td>
<td>-2.1</td>
</tr>
<tr>
<td>hsa-miR-200b-3p</td>
<td>0.001</td>
<td>0.008</td>
<td>0.828</td>
<td>-3.7</td>
</tr>
</tbody>
</table>

- 6 miRNA downregulated in LS with fold change <-2
Hierarchical clustering analysis resulted in two distinct clusters differentiating LS and non-LS samples.
Results
Gene Ontology

For the top eight differentially expressed miRNA (FDR<0.001 and a FC of <-2 or >2), 58 significant biologic processes were predicted.
Clusters 1-4 indicate an immune response component differentiating these cohorts; cluster 5 represents processes involved in wound healing, primarily angiogenesis and fibrosis.
Discussion

- First study evaluating USD pathophysiology with miRNA expression profiles in LS and non-LS USD

- miRNA identified regulate gene expression in pathways responsible for inflammation, autoimmunity, systemic sclerosis, cell proliferation, apoptosis, and angiogenesis
Discussion

miR-155-5p

- Frequently implicated in the tumorigenesis and progression of multiple types of cancers including colorectal and gastric carcinoma

- Studies of vulvar LS tissue samples found miR-155-5p to be significantly upregulated
  - Involved in fibroblast cell proliferation by inhibition of FOXO signaling pathway

- Implicates miR-155-5p as a potential therapeutic target for vulvar LS; our results suggest a potential role in LS USD
Discussion

Other miRNAs

- miR-146a-5p considered to act as a tumor suppressor in prostate and gastric cancer

- Urinary levels of miR-146a-5p were found to be elevated in bladder cancer patients and were associated with tumor grade and depth of invasion

- miR-150-5p has been shown to also act as a tumor suppressor in prostate cancer
Levy et al. examined protein expression in LS and non-LS USD and found significantly higher levels of inflammatory markers in strictures caused by LS including CD8 T cells and CCL-4.

In our study, the biologic processes predicted of the miRNA involved support the theory of inflammatory and possible immune components.
Discussion
Inflammation and Immunity

- Innate immune response implicated, primarily involving several of the toll-like receptors

- These signaling pathways are essential for the skin’s inflammatory response against invasive pathogens

- Over-activation often leads to uncontrolled inflammation and then development of autoimmunity and/or inflammatory skin diseases
Discussion

Limitations

- Exploratory study using miRNA as a surrogate for gene expression

- All samples used in this study represent a highly selected population

- Although sample size for this exploratory study is small, it does not invalidate the profound differences found between the study cohorts
Discussion

Implications and Future Directions

- Differentially expressed miRNA identified in this study could serve as biomarkers of LS
  - Aid urologists and pathologists in diagnosis, treatment, and possible prevention of LS USD

- miRNA in situ hybridization could be a diagnostic modality used to better classify suggestive lesions that don’t reach definitive histologic criteria to be diagnostic of LS

- Urinary miRNA evaluation could potentially serve as a non-invasive method for evaluation of USD
Conclusions

- Novel investigation of miRNA expression profiles in LS and non-LS USD

- LS urethral strictures demonstrate differential expression of miRNA involved in processes responsible for inflammation and immune response when compared to non-LS USD

- miRNA identified in this study suggest excellent predictive value for distinguishing LS vs non-LS USD samples and could potentially serve as biomarkers of LS with further validation in larger cohorts
Acknowledgements

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