

# Discovering non-coding RNAs involved in spatial arrangement of fibroblasts spanning the human body

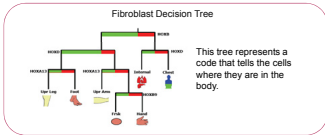
Nouran, Supriya Gupta, Howard Rafal, Mitchell Guttman and John Rinn



## Introduction

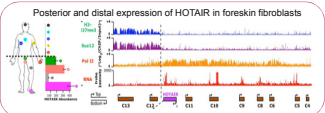
A major issue in developmental biology is understanding how differential gene expression gives rise to the spatial arrangement of the different organs in the body. The human skin represents exceptional differences in its structure and function across anatomic sites. This raises many ongoing questions on how the cells maintain their positional information in this complex, self-renewing tissue. In addition, the skin consists of fibroblast cells that play a major role in encoding positional identity to epithelial cells. Previous studies have shown that fibroblasts from different parts of the body have unique gene expression patterns and could therefore help specify the developmental and spatial organization of many cell types.

Fibroblasts from different parts of the body express different genes based on their location relative to three anatomic divisions: anterior-posterior, proximal-distal, and dermal-non-dermal. A large set of genes were discovered that are differentially expressed between fibroblasts from different positions in the body. These studies focused on protein coding RNAs – transcripts that code for proteins, many of which are of known function.



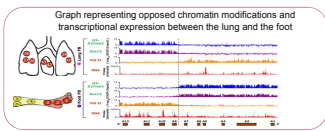
Further analysis pointed to HOTAIR, playing an important role in positioning. HOTAIR is a large intergenic noncoding RNA (lincRNA). lincRNAs are transcripts that are not translated into proteins but they can influence gene expression by interacting with other regulators. HOTAIR was found to be one of the genes that is expressed only in the posterior and distal locations of the body. This implies that it is expressed in foot, fingers and forebrain. HOTAIR was discovered to be the first example of a noncoding RNA that regulates chromatin domains.

It has further been identified that HOTAIR is involved in the methylation and transcriptional regulation of HOX genes (genes that are regulated during embryonic development and describe the positional identity of the cell or tissue). Therefore, it is one of the genes that identifies the positional arrangement of cells. These findings led to the possibility of other lincRNAs existing that might be correlated with the spatial organization of tissues.



The purpose of this study is to determine if additional non-coding RNAs are differentially expressed in fibroblasts from different regions of the human body. Our specific goal is to identify non-coding RNAs that may provide positional information to fibroblasts.

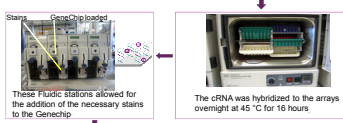
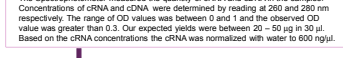
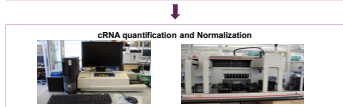
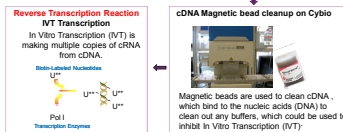
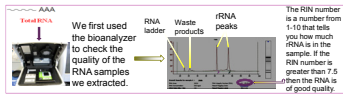
To achieve our goal, we compared the gene expression profiles of cells from different locations of the body. To expand on the previous work, we used arrays that contain probes of a larger gene set, which includes genes for non-coding RNAs. We analyzed a series of samples, but chose to focus on lung and foot, as they have been found to be diametrically opposed in their gene expression profiles.



## Methods

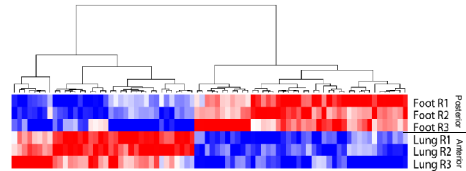


RNA from fibroblast cell lines was extracted and used as our samples. We chose 4 different anatomical sites to analyze: lung, forebrain, foot and scalp. Total RNA extracted from cells is processed for hybridization on the Genechip arrays using the Affymetrix one cycle target preparation assay.



## Results

### 79 non coding RNAs are differentially expressed between lung and foot



These lincRNAs could be involved in determining positional information and regulation of biological pathways. We decided to look at the functional gene sets that these lincRNAs associate with, using Gene Set Enrichment Analysis.

### What do these 79 lincRNAs do?

We focused on positional identity gene sets. These are protein coding genes previously identified to be differentially expressed between different positional locations.

We discovered interesting correlations between the previously known protein coding genes and our newly identified and noncoding RNAs. An example of this similarity is apparent between HOXA13 and the lincRNA. Interestingly HOXA13 was previously found to be a master regulator, which acts in the activation of the positional information of the posterior-distal locations of the body.

Almost all of the lincRNAs we identified significantly (FDR < 0.05) associate with protein-coding genes known to be involved in positional identity.

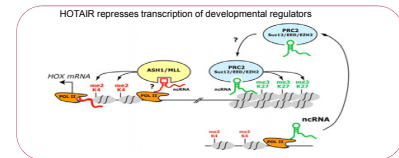
Surprisingly, the few that do not associate with positional identity include a well-known lincRNA, XIST. XIST is a lincRNA known to be differentially expressed between males and females. Consistent with this, our lung sample came from a female and our foot sample came from a male. This suggests that these few lincRNAs that are not involved in spatial arrangement might be involved in sex-specific differences.

## Conclusions

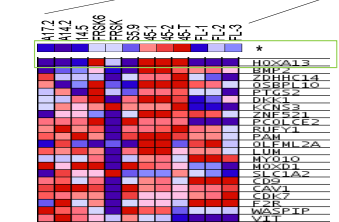
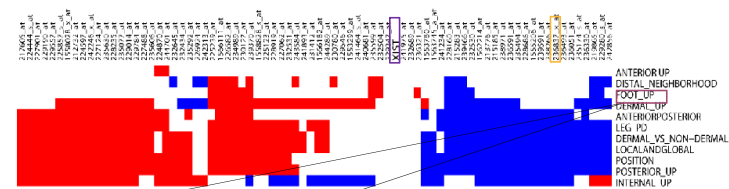
After successfully completing this research, we can conclude that noncoding RNAs are an extra layer of regulation that play an important role in identifying positional information.

When the lincRNAs were correlated with the protein coding genes from different positions of the body, we were able to see the similarities they share. Since we know the functions of the protein coding genes, we could infer some of the roles that the lincRNAs play in identifying spatial arrangement. In the future, we can knock out the lincRNAs to elucidate the function they play in fibroblast positioning. This will allow us to determine which genes these lincRNAs regulate and potentially discover master regulators.

In addition, similar to the HOTAIR discovery, each of the 79 noncoding RNAs may be further analyzed for their functions. This will be done by knocking out each lincRNA down, which allows us to determine their functions and regulatory mechanisms. This will allow us to determine if each ncRNA functions in a mechanism similar to that of the known lincRNA HOTAIR. In this model, the lincRNA binds to the PRC2, which inhibits the transcription of the HOXD gene.



### Expression patterns of these ncRNAs correlate with those of known protein coding RNAs from different locations of the body

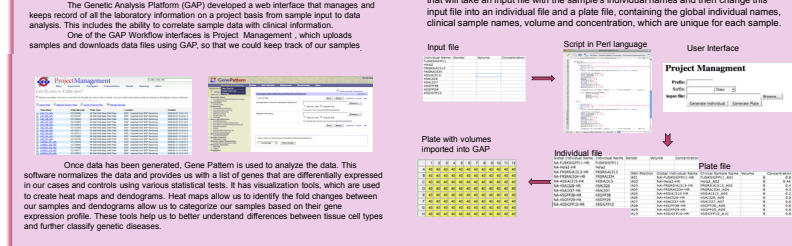


\* An example of a lincRNA that is expressed in many locations of the foot

The protein-coding genes that show a similar gene expression profile to a non-coding RNA in foot fibroblasts

Key:  
■ Genes that are upregulated  
■ Genes that are downregulated

## Overview of Informatics Workflow



## Citations

**Fibroblasts**  
[http://www.immiedart.com/catalog/images/bg\\_images/SPL\\_6\\_p780110-Fibroblast\\_cells\\_showing\\_cytoskeleton.jpg](http://www.immiedart.com/catalog/images/bg_images/SPL_6_p780110-Fibroblast_cells_showing_cytoskeleton.jpg)  
**Embryo**  
<http://www.biology.lsu.edu/heydrjy1202/Chapter24/Hox%203.jpg>  
**Da Vinci Man**  
<http://www.meclectinc.com/DiVinciManET.gif>

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