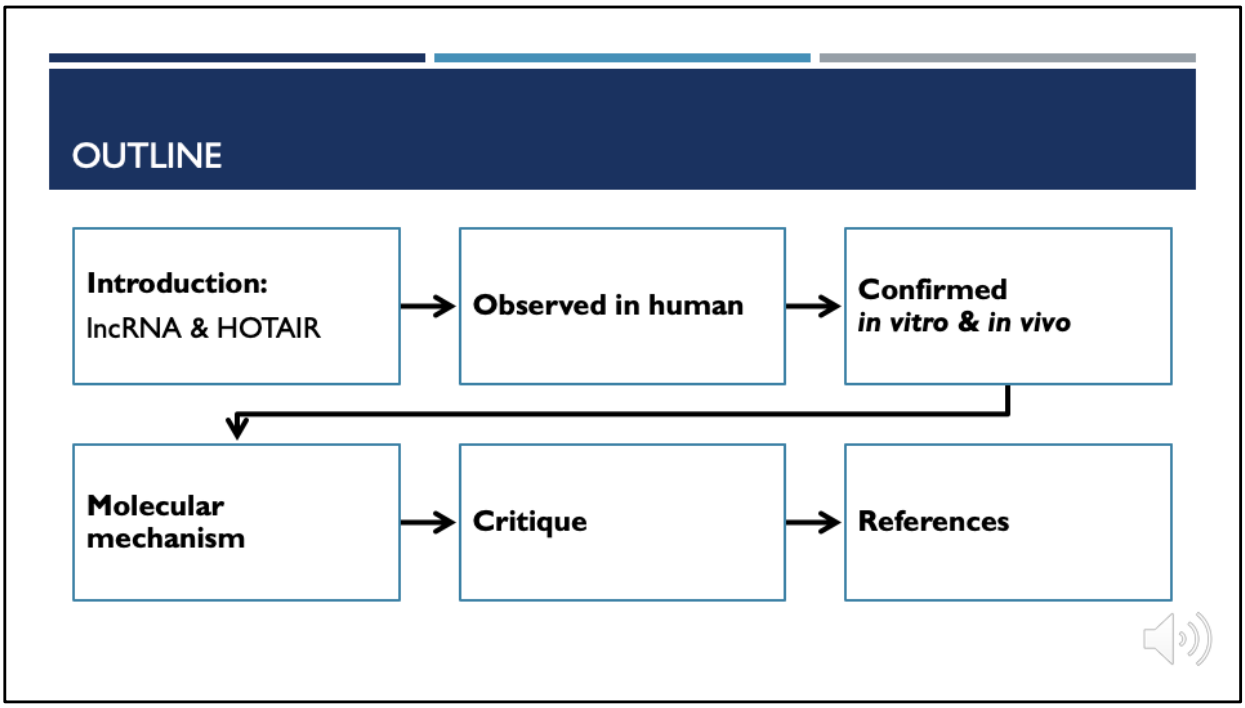
Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis

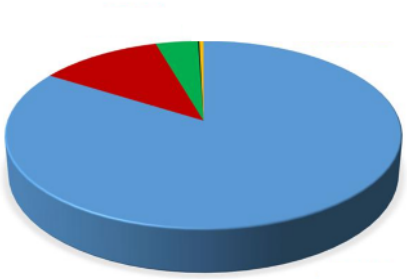
Gupta, et al. (2010). *Nature*, *464*(7291):1071–1076.
*Corresponded by **Howard Chang** at Stanford

Hi, I'm Yaoding. Today, we are going to talk about a paper published on Nature in 2010, titled Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. The paper is corresponded by Howard Chang at Stanford, who is a leading figure in epigenetic study of long non-coding RNA.



First, I will show you the outline of the presentation, which starts with an introduction to both lncRNA & HOTAIR. Then, we will talk about the results in three sections, including the observations and analysis based on data from human, then evidence from cellular and animal studies, finally the molecular mechanism revealed by the study. We will talk about the method during the results and critically evaluate the results in critique section. We will have a references section to end the presentation.

Introduction:
What is long non-coding RNA (lncRNA) ONG



RNA by mass

Definition:

- **Long**: > 200 nucleotides
- Not translated into protein

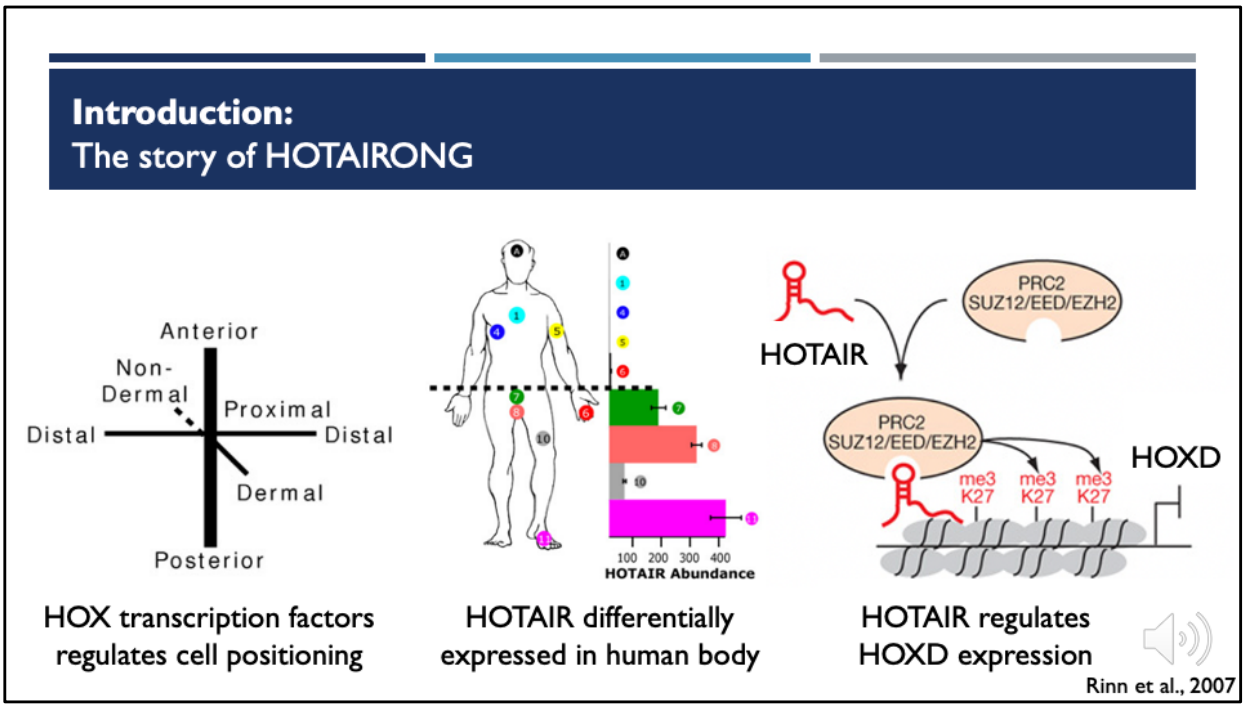
Significance:

- **Conserved** across mammals
- **Regulating** gene expression

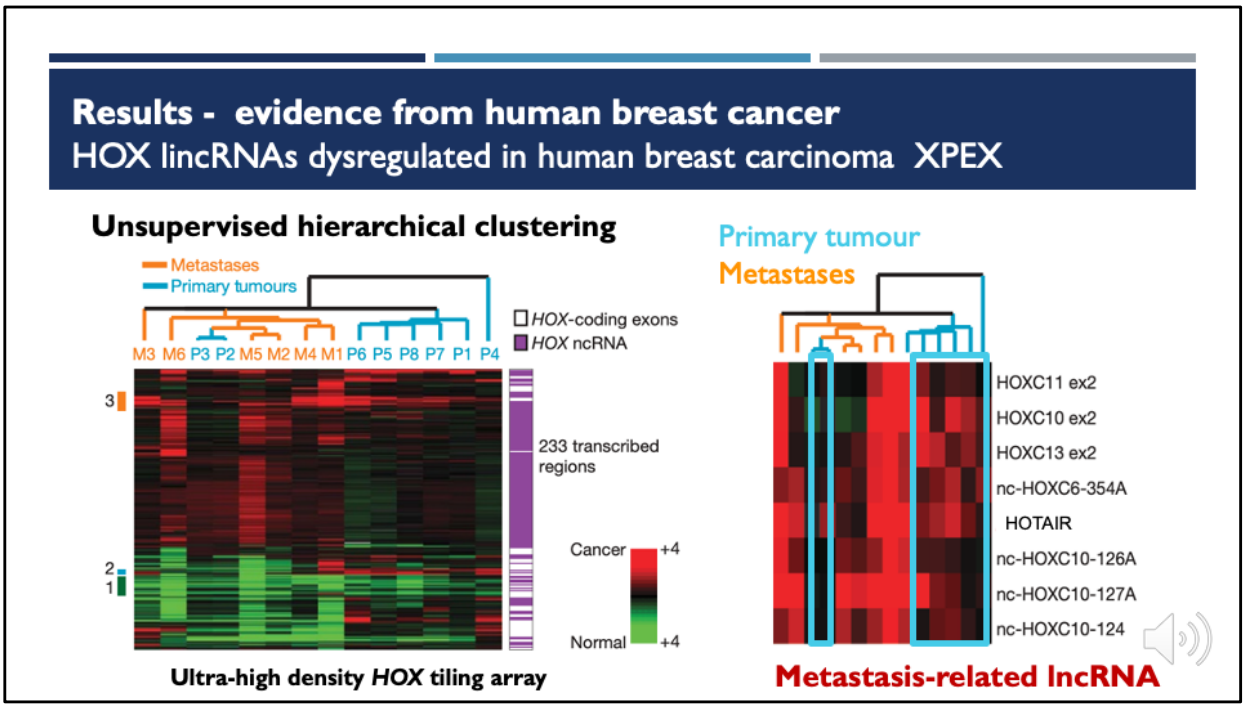
HOTAIR

Palazzo & Lee, 2015

The central dogma tells us genes are transcribed into RNA and then RNA is translated to protein. RNA plays an important role. But protein-coding mRNA only account for 8% of the total RNA mass, while most of the RNA are non-coding. A tiny proportion of the RNA is called long non-coding RNA, lncRNA, which individually has more than 200 nucleotides. Many of them are highly conserved across mammals, with unknown functions. HOTAIR is one of the rare known to epigenetically regulate gene expression.



The story of HOTAIR starts with the research into HOX transcription factors, which are essential for the correct positioning of the cell. HOTAIR is expressed differentially in positions where HOX transcription factors are present. Previous study has shown that HOTAIR, which resides in HOXC locus, can bind to PRC2 complex to induce H3 lysine-27 trimethylation of HOXD locus. This is the first non-coding RNA to regulate the transcription in another chromosome.

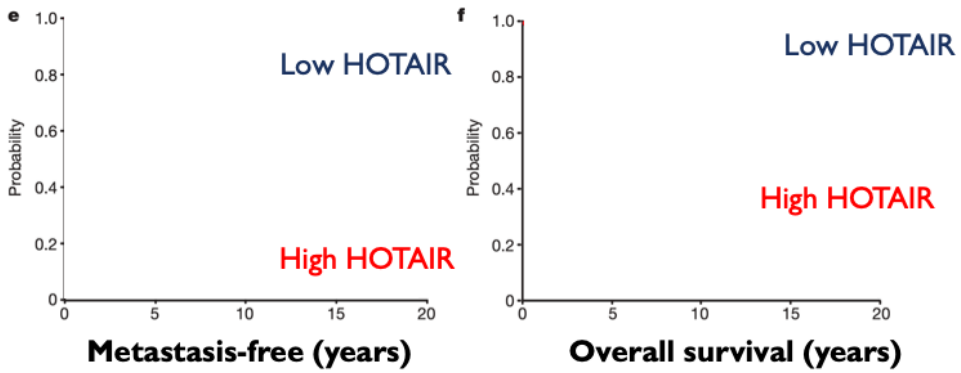


HOTAIR could have a regulatory function, but what is the phenotype of the regulation? We know that HOX expression is often dysregulated in breast cancer, so we figure that there might be a role of HOTAIR.

To study this, the authors designed an ultra-dense *HOX* tiling arrays which can quantify the transcription level of all transcribed region in HOX, with probes carrying cy3 and cy5 dyes labelling normal and cancer cells respectively. According to transcription level measured by the microarray, the authors used computer to hierarchically cluster clinical samples. With clustered samples, we can find the relationship between different tumour stages and transcription level. The results shows a series of ncRNA that are are rare in primary tumour but abundant in metastatic tumour. Among them is HOTAIR.

Results - evidence from human breast cancer
High HOTAIR expression is associated with poor prognosis

Previous clinical study: 1/3 of the patients with 125X higher HOTAIR expression



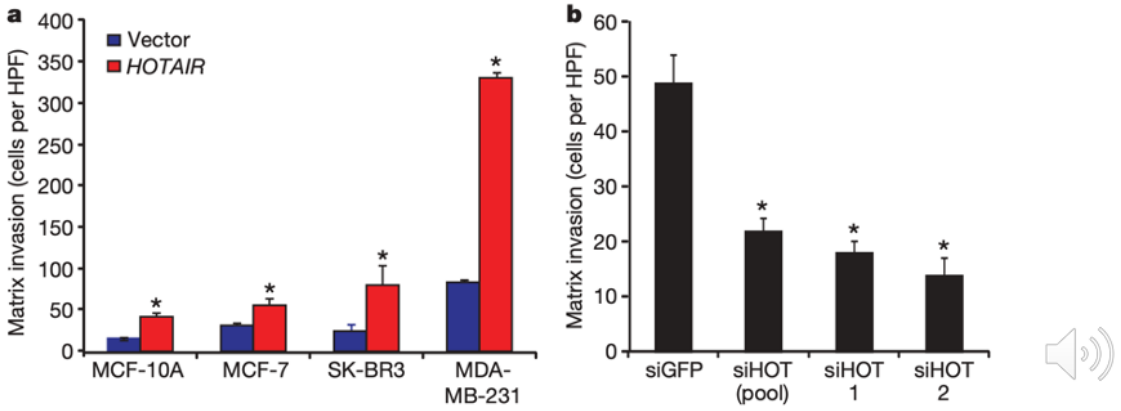
Then is the association between HOTAIR level and cancer stages real? To answer this, the authors mined the data of a previous clinical study, finding that 1/3 patients has 125 times higher HOTAIR expression. The higher expression level is found to be associated with worse prognosis, indicated by shorter metastasis-free time and overall survival time in the follow-up study.

Results - evidence from cell and animal studies

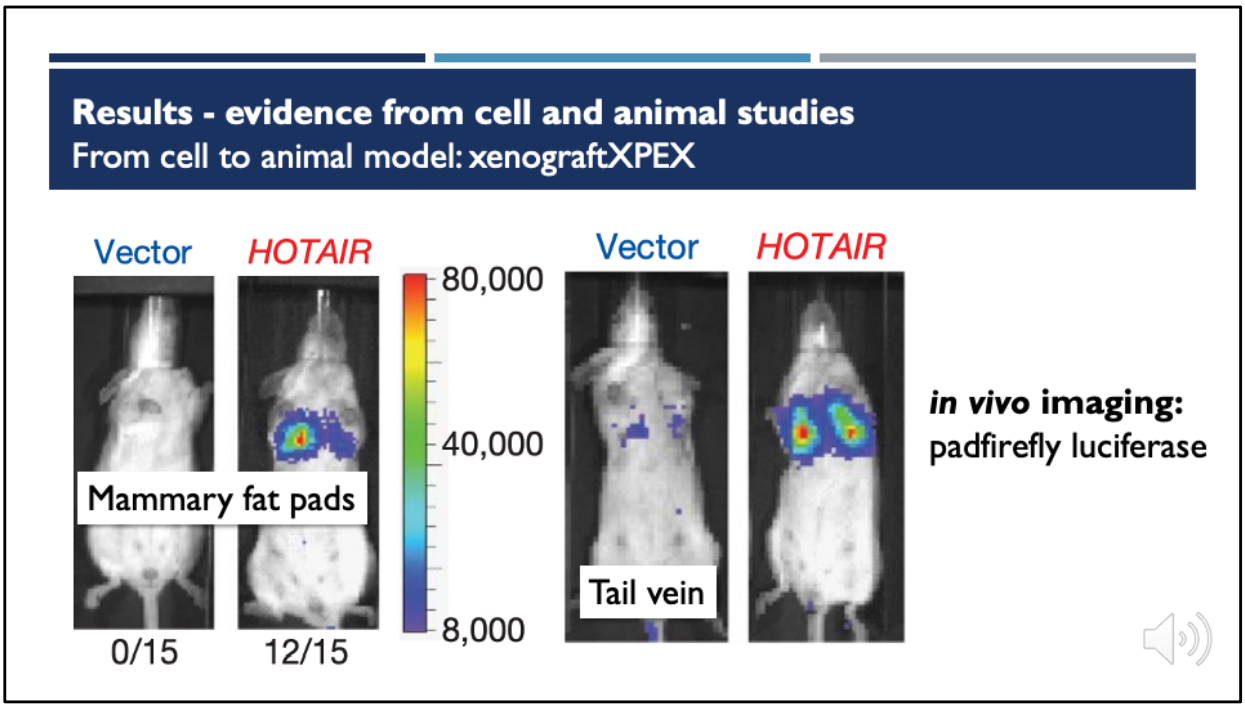
HOTAIR overexpression promotes matrix invasion in breast cancer cellsXPEX

HOTAIR overexpression through retroviral transduction promotes invasion

HOTAIR silencing via siRNA reduces invasion

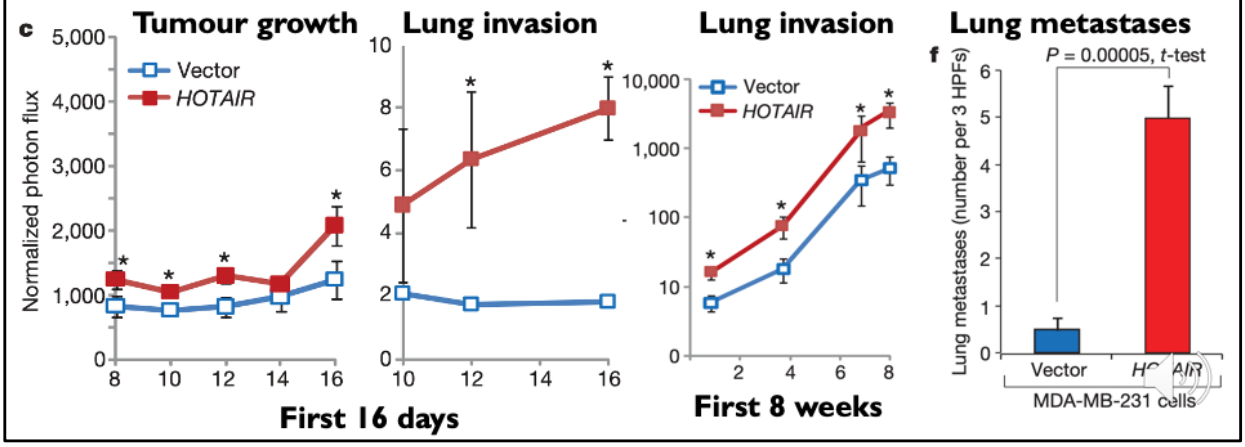


The association is also confirmed in vitro with different kinds of beast cancer cell. High HOTAIR level significantly increases the matrix invasion in all breast cancer cell lines, whereas silencing of HOTAIR with siRNA reduces the invasion.

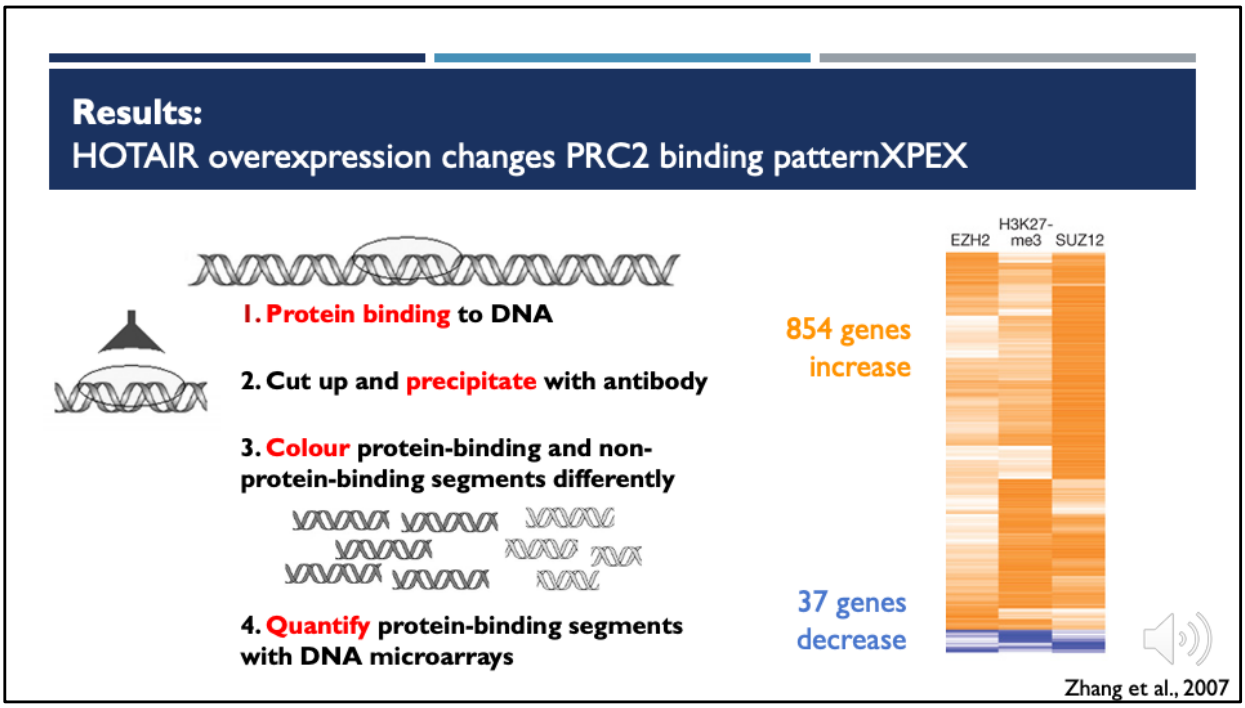


To further test the effect in vivo, they use a mouse model. They transfected the cancer cell lines with padfirely luciferase so that we can visualise the tumour growth and quantify the cell number according to the light intensity. As is shown, after xenografted with overexpressed HOTAIR in mammary fat pads and tail vein, the mice had cancer cell proliferation around the breast.

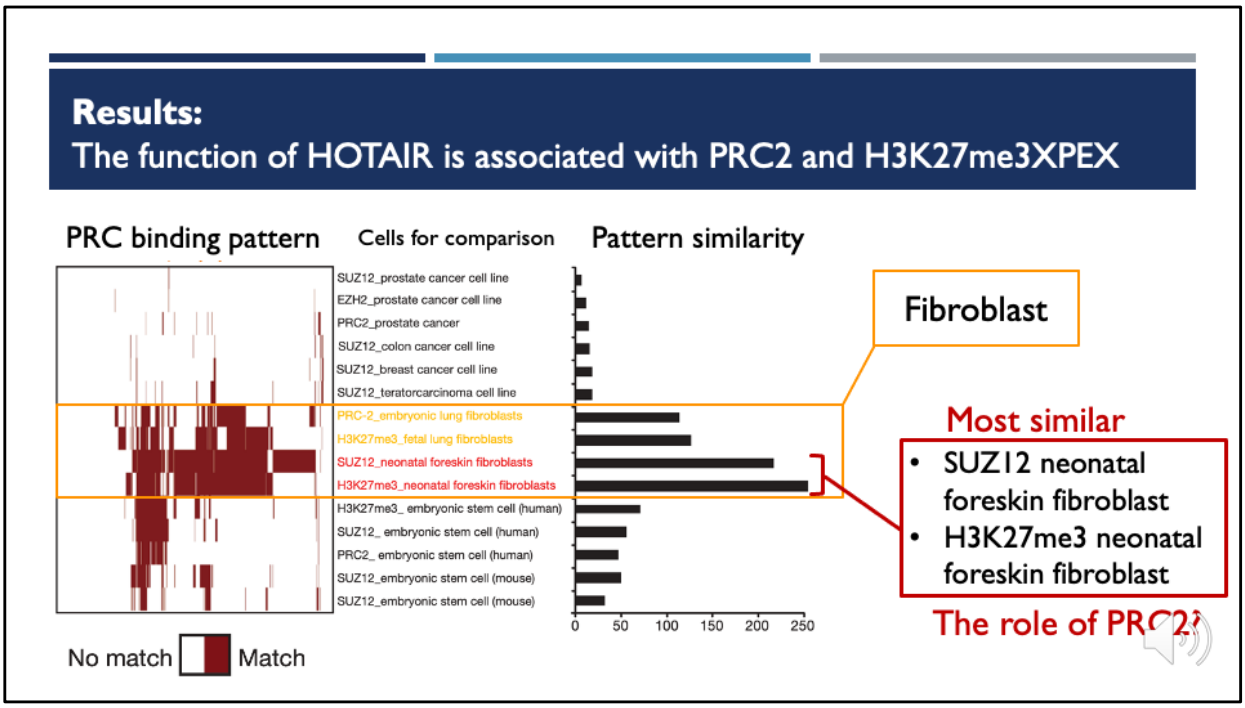
Results - evidence from cell and animal studies
HOTAIR overexpression promotes lung invasion and metastases



The transplantation into the mammary fat pads, apparently, prmotes the primary tumour growth and maxtrix invasion. Even though transplanatation was made in tail vein, which is far from lungs and mammary glands, tumour cells with overexpressed HOTAIR still causes more lung invasion and metastases, as shown in two figures on the right.

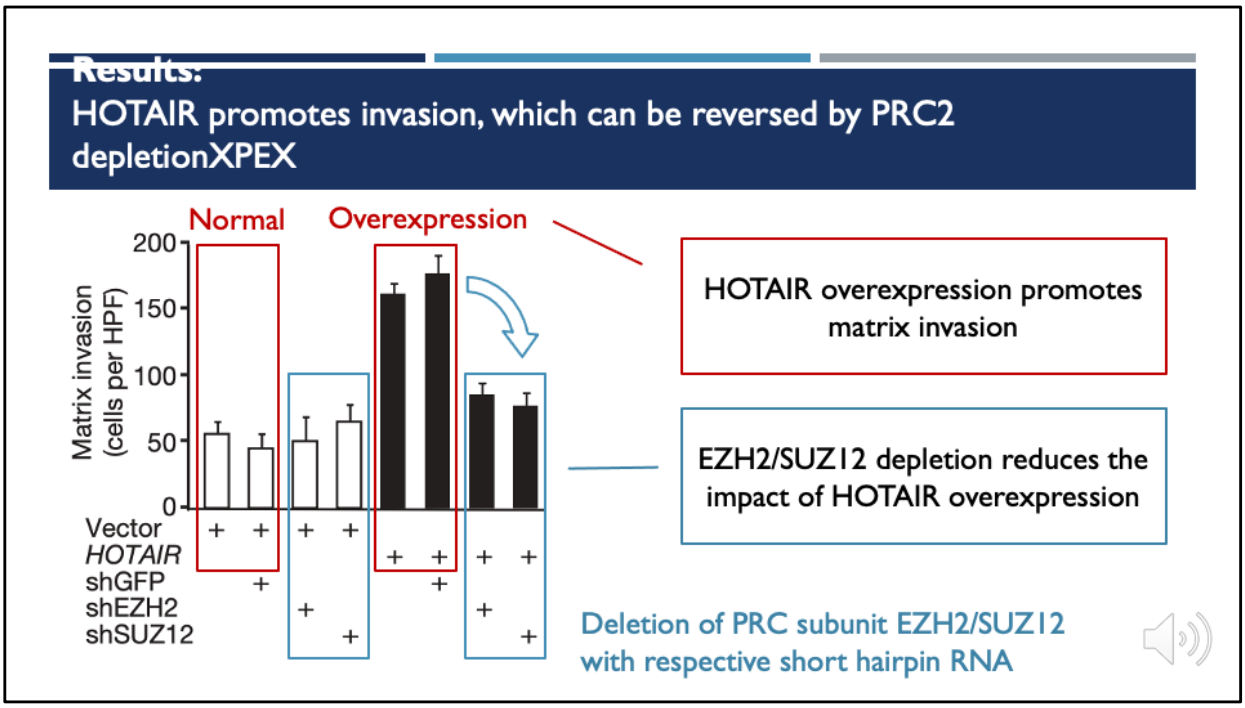


Since HOTAIR is associated with epigentic regulation thourgh PRC2, we need to how PRC2 binding pattern changes. To do that, the authors used Chip-Chip which uses chromatin immunoprecipitation to specifically bind and precipitate the chromatin slice binding with PRC2, and then protein microarray to quantify the binding and non-binding sites, as is shown in the illustration on the left.
They found that there are 854 genes with increased PRC2 occupancy and 37 genes with decreased occupancy. This shows that PRC2 occupancy pattern is changed by high HOTAIR.

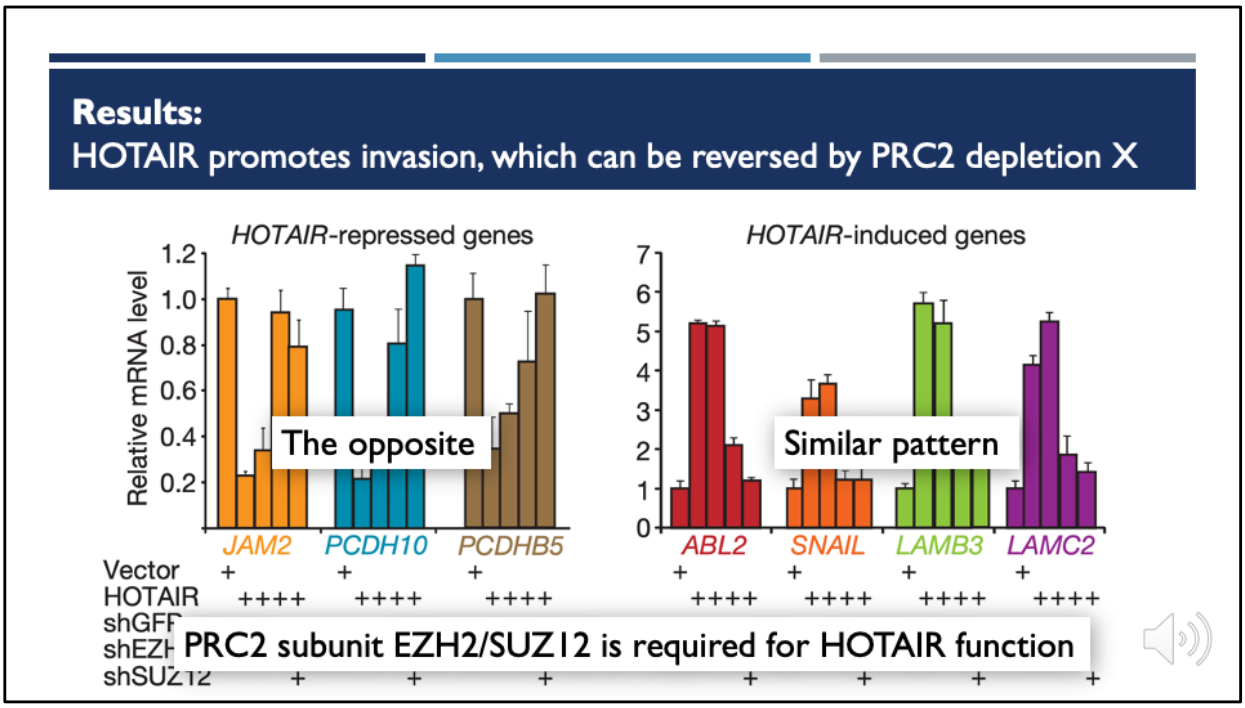


By comparing the new pattern with ever-known pattern in other cells, we can know what the new pattern could mean. In the left panel here, we compare the PRC occupancy state of our Chip results with that of other cells. If they have the same occupancy state for each gene, then it will be a red match, or else a blank ”no match.” Among all the cells we compared, 4 fibrablasts have the highest occupancy similarity, meaning that high HOTAIR reprograms the chromatin state of a breast epithelia to that of a fibroblast-like cells.

Among them, fibroblast with PRC2 subunit SUZ12 and H3K27me3 modification, which is a known result of HOTAIR/PRC mediated epigenetic regulation have the highest similarity among 4 firbroblast, suggesting the importance of PRC2 in the process.

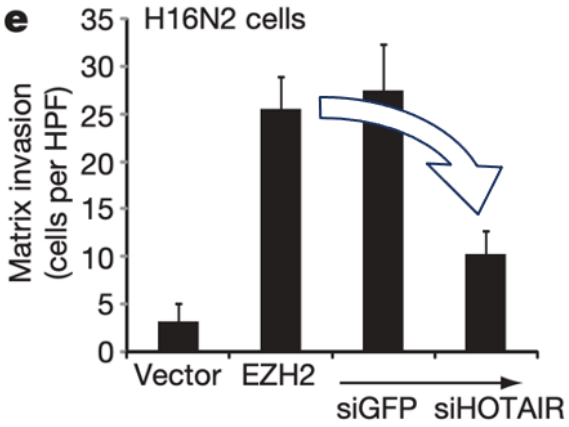


So, we test whether PRC2 is required for the HOTAIR function. First, HOTAIR overexpression can promotes matrix invasion, as is shown in red boxes. After we depleted PRC subnits EZH2/SUZ12 with shRNA, overexpression of HOTAIR does not seem to affect the matrix invasion level, as shown in blue boxes. Also, we found on the right of the figure that PRC2 subunit depletion reverses the effect of HOTAIR, which indicates that a complete PRC2 structure is required for HOTAIR function.



The pattern is basically the same for HOTAIR-induced genes. The pattern would be the opposite for HOTAIR repressed genes. But what is not changed is that the reversal of HOTAIR effect by depletion of PRC subunits EZH2/SUZ12 is also observed in mRNA levels.

Results:
EZH2 promotes invasion, which can be reversed by HOTAIR depletion X

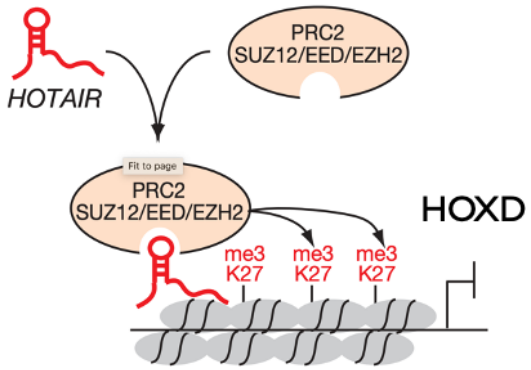


HOTAIR is required for PRC subunit EZH2 function

Gene silencing with siRNA

Interestingly, overexpression of PRC2 subunit EZH2 can also increase the matrix invasion, while depletion of HOTAIR with short interfereing RNA can reverse the change, which shows that HOTAIR is essential for PRC subunit EZH2's function to promote cancer progression. That is all our major results.

Critique: background information X

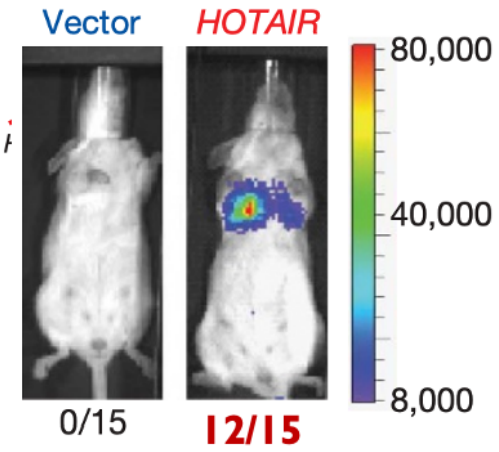


- **Introduction**
  - HOTAIR in HOXC said to regulates HOXD transcriptionally, but this is controversial…

Amandio et al., 2016 ; Selleri et al. , 2016

What features the study is that it proposed that HOTAIR can promote oncogenesis with the same mechanism as it does to HOXD. But since 2016, there is controversy over HOTAIR function, because some of the phenotypes claimed by Chang's lab cannot be reproduced even with the mice provided by Chang's lab.

Critique: background information X



- **Figure 2d**

  HOTAIR controversy:

  What happens to the 3 mice without metastasis?

Also, we found in this study, there are also 3 mice without effective effect of HOTAIR. One possible reason might be that there are other regulators of HOTAIR that we don't know.

Critique: experimental designX

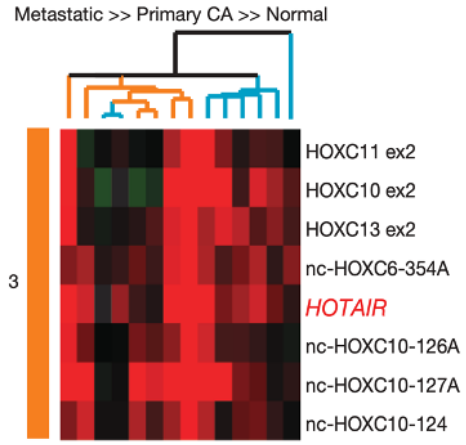


Figure 1b:

- Why only HOTAIR is studied?
- What is the function of other lncRNA?

Another question is that we find a series of lncRNA associated with cancer stages, but only studied HOTAIR. Then, what is the role of other lncRNA? I don't think we have enough research to answer this so far.

Critique: experimental design X

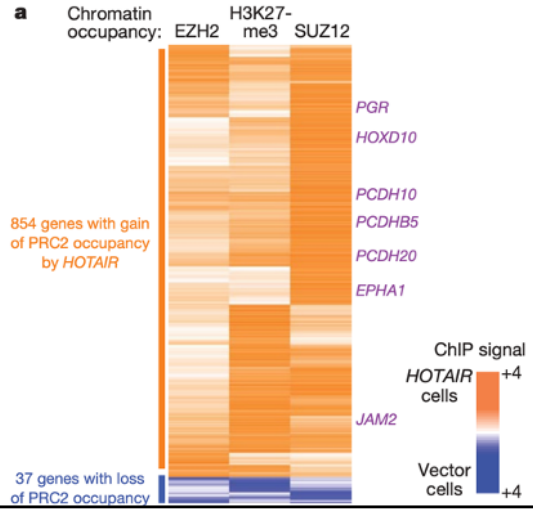


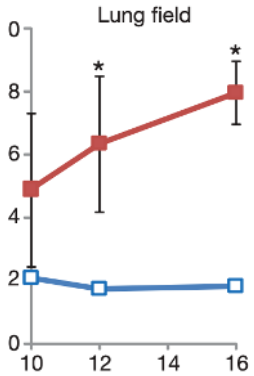
Figure 3a

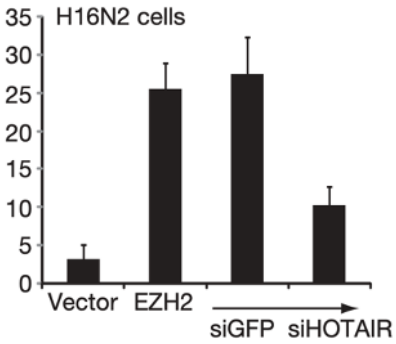
High HOTAIR changes the occupancy pattern, but how each specific location's occupancy is changed?

Chip-chip vs. Chip-seq

For Figure 3a, we can see that high HOTAIR changes the PRC binding state of nearly 900 genes, but how each of the gene's occupancy state is still unclear. I think there should be a complex signal casade to induce all these changes. Also, note that today we can use Chip-seq to have a more accurate measurement chromatin marks today, since I have learnt how to do this in some ZJU labs.

Critique: data presentationX





- Missing error bar?
- Missing data? How about 1-10 days and 14th day?
- Missing p-value?

Figure 2C (right panel) **Figure 4e and others**

There are also problems with data presentation. In the right panel of figure 2C, I can't see an error bar for the blue line. I think it is missing. Also, the authors do not present the data of 14th day and the data points are quite few actually. For some facets in Figure 4, for example, Figure 4a, there is no p-value calculated for comparison. I don't think it will be better for us to compare the values totally by looking at the data.

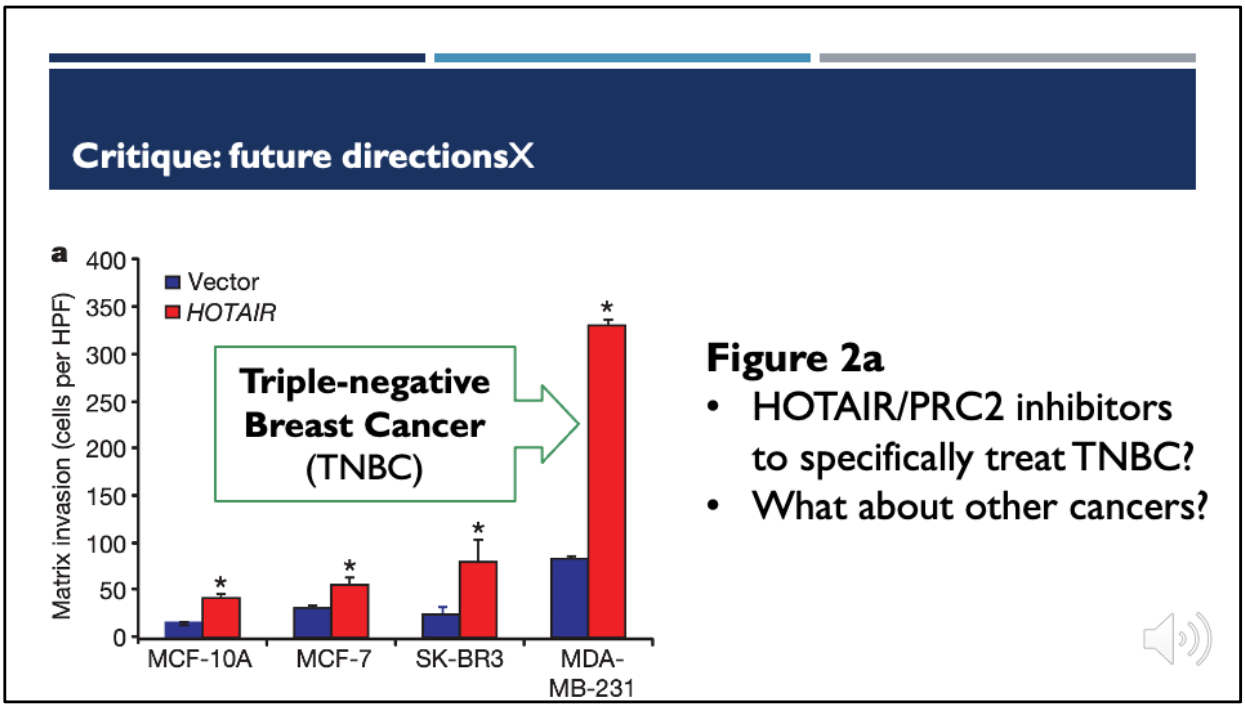


Figure 2a shows that HOTAIR has much more impact on matrix invasion in MDA-MB-231 cell line which is a triple-negative breast cancer which lacks targeted therapies. This study provides a hope for HOTAIR/PRC2 inhibitors to treat TNBC. We need to investigate into whether this is effective and whether it can be applied to other cancer types.

Further reading on module map
Segal lab of computational biology. *Create a Module Map: Characterize Expression Data Using Gene Sets*. Available at: https://genomica.weizmann.ac.il/Tutorial/create_module_map.html

Further reading on lncRNA
Howard Y. Chang. *Genome regulation by long noncoding RNAs*. Available at: https://www.youtube.com/watch?v=xAYXE-iplKk

THANKS FOR WATCHING!

ReferencesEX

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