# dFOXO, a novel regulator of stress inducible Hsp70, drives Hippo-mediated tumorigenesis

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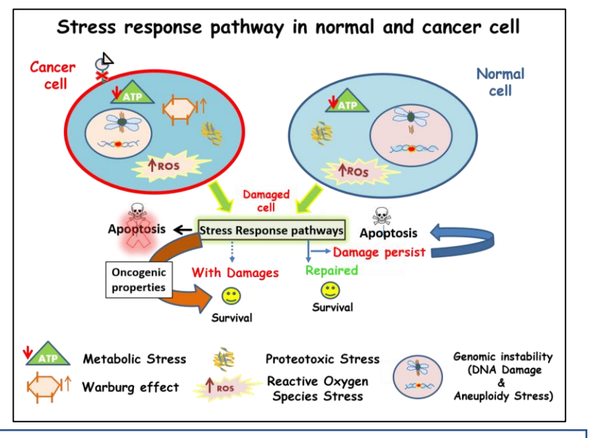
## Introduction & Motivation

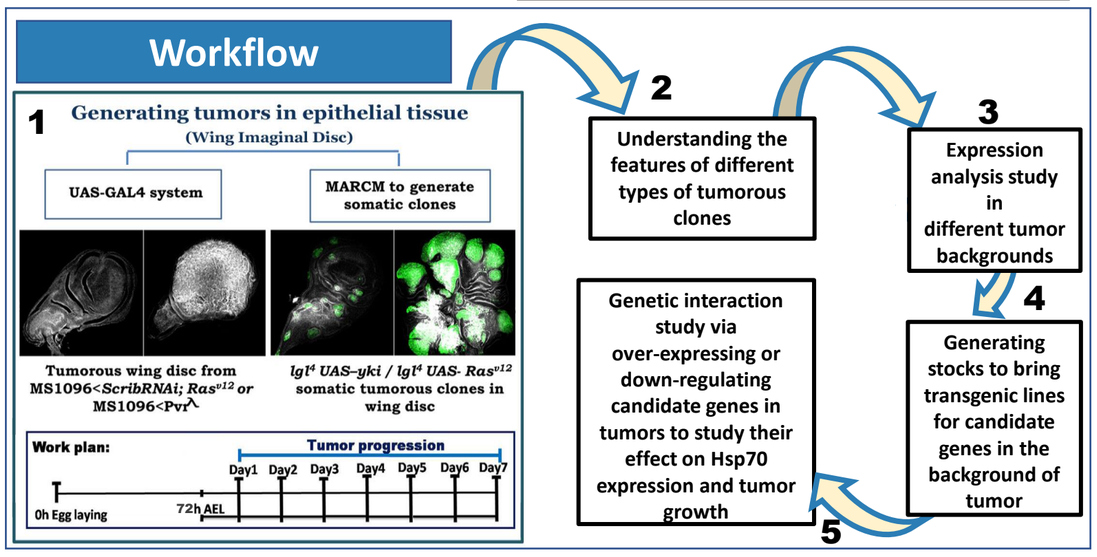
Cancer cells face tremendous stressful conditions during tumorigenesis. Adaptive re-wiring of cellular signaling pathways in cancer cells facilitates a robust stress response for their survival in the hostile milieu. Heat shock proteins(Hsps), major regulators of stress response are known to be overexpressed in different human cancers and are implicated in tumorigenesis. Expression of Hsps is believed to be necessary for tumor cells, but the molecular basis of Hsp dependent oncogenic properties and their regulatory mechanisms are yet not clear and also little is known about their role in early stages of cancer development. In order to gain insight, we looked into expression profile of different Hsps in different tumor backgrounds. Interestingly we found Hsp70, key protein of stress response pathway, was expressed only in a subset of tumor cells in different tumors. As Hsp70 is exclusively stress inducible and plays crucial roles during different stress conditions we undertook this study to look into the significance of this expression pattern and dissected the molecular basis of its regulation.




Important facts:

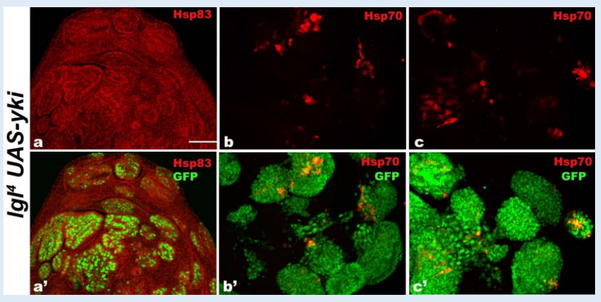
1. Hsp70 is critical for tumor development, downregulation of Hsp70 gene inhibits tumor cell metastasis in mice. (Gong et al., 2015)
2. HSF1, master regulator of Heat shock proteins regulates molecular chaperones HSP90 and HSP70 in tumor biology (Jin et al., 2011; Solimini et al., 2007).
3. dFOXO as an emerging regulator of Hsp70 (Donavan and Marr 2016, Jensen et al., 2017).
4. FOXO transcription factors have been majorly considered as tumor suppressors, yet few cancers study report its role in metastasis. These reports highlights its complex role in tumor development (Coomans and Demoulin 2016).

## Workflow



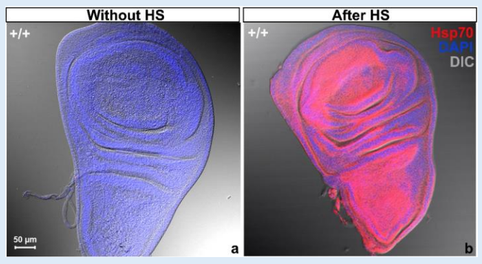

## RESULTS

### 1. Expression of Heat shock proteins in tumors



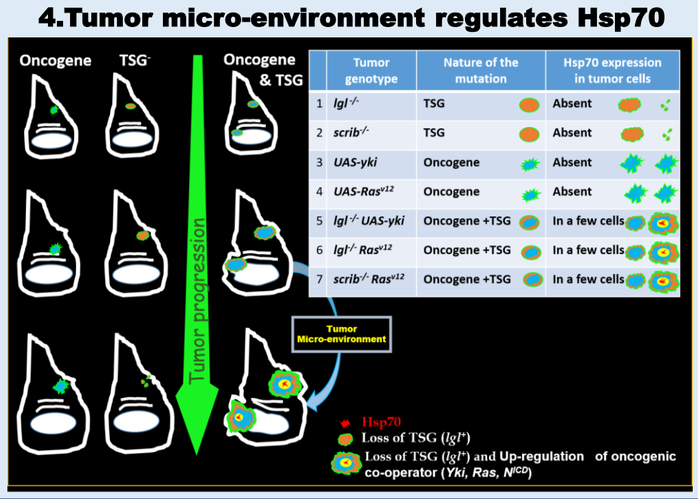

**Unlike other Heat Shock Proteins (eg. Hsp83), Hsp70 is expressed in a subset of tumor cells.**

### 2. Expression of Hsp70 in wing disc:



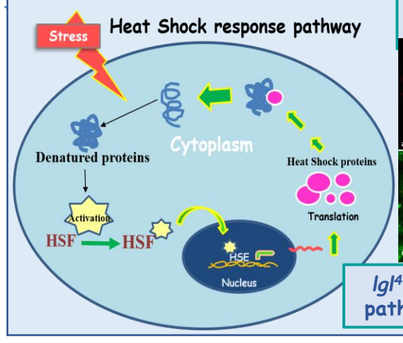

Low level of transcripts are expressed during development but its protein is reported to be exclusively stress inducible

### 4.Tumor micro-environment regulates Hsp70

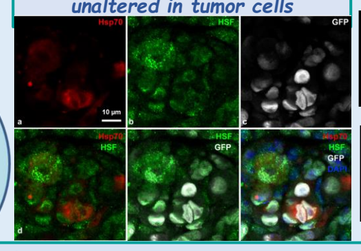


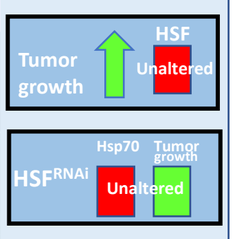

| | Tumor genotype | Nature of the mutation | Hsp70 expression in tumor cells |
|---|---|---|---|
| 1 | lgl$^{-/-}$ | TSG | Absent |
| 2 | scrib$^{-/-}$ | TSG | Absent |
| 3 | UAS-yki | Oncogene | Absent |
| 4 | UAS-Ras$^{v12}$ | Oncogene | Absent |
| 5 | lgl$^{-/-}$ UAS-yki | Oncogene +TSG | In a few cells |
| 6 | lgl$^{-/-}$ Ras$^{v12}$ | Oncogene +TSG | In a few cells |
| 7 | scrib$^{-/-}$ Ras$^{v12}$ | Oncogene +TSG | In a few cells |

### 5. Hsp70 is regulated via non-canonical, HSF independent pathway




*HSF expression remained unaltered in tumor cells*



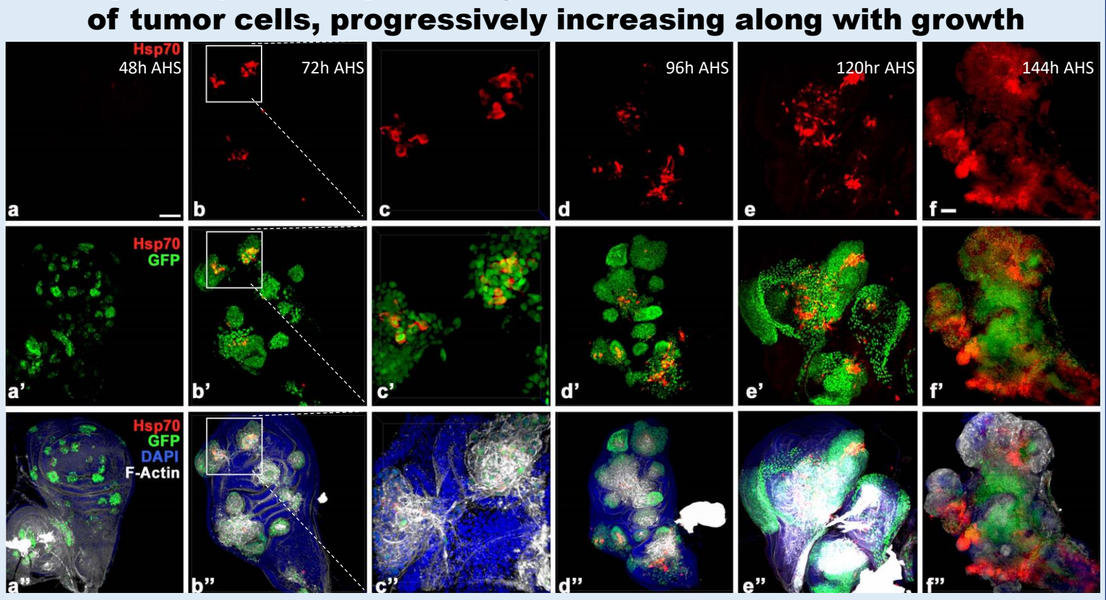




*lgl$^{4}$ UAS-yki* clones show HSF independent growth; Hippo pathway mediated tumors are independent of HSF activity

Study of Hsp70 expression in different genotypes

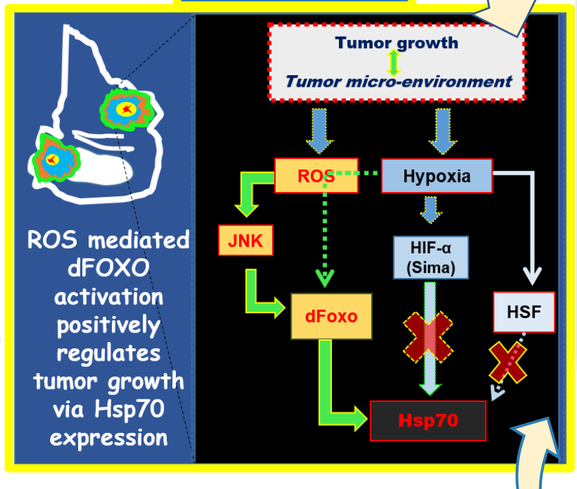
- Why Hsp70 expression was limited to a few tumor cells; How is it regulated?
- How these handful Hsp70 positive cells impact tumor growth?

### 3. Hsp70 expression positively corelates with transformation rate of tumor cells, progressively increasing along with growth

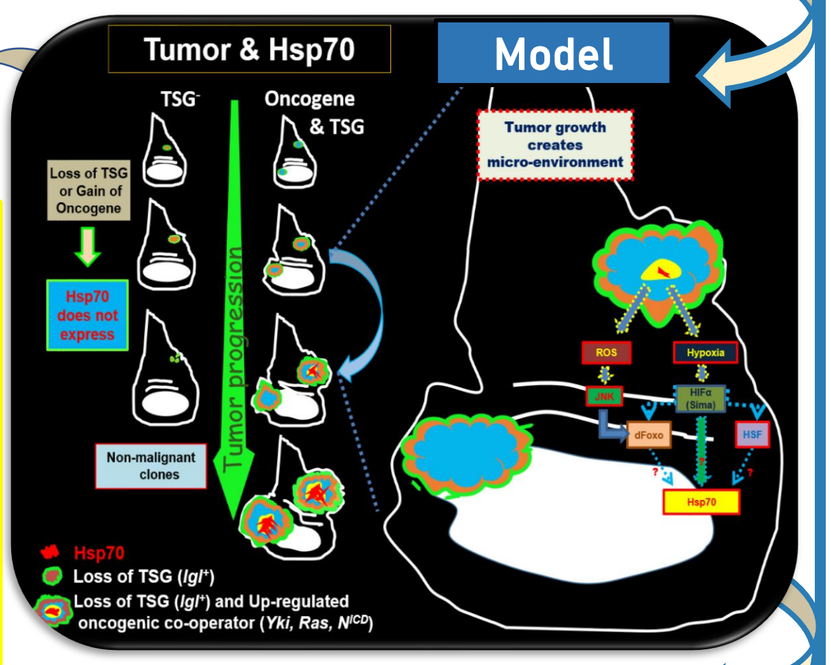



Hsp70 regulates tumor growth


## Conclusion

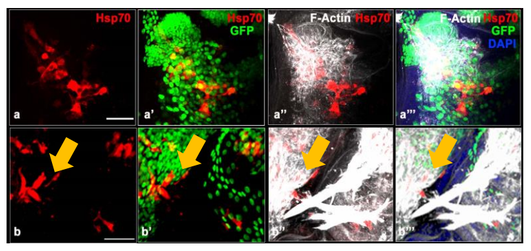



## Model



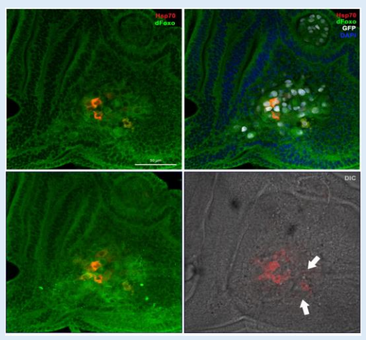

### Future directions:

Why do Hsp70 expressing cells show mesenchymal properties?




- Hsp70$^{+ve}$ cells have elongated extension (a-b''') and are often present at F-actin nucleation points. b-b''' show magnified area of inter disc connection from 3.e-e'''
- It's down-regulation reduces the no. of cells having mesenchymal features, indicating probable role in cytoskeleton regulation

### 6. dFOXO is crucial for Hsp70 expression and tumor growth




*Increased dFoxo expression in the niche having Hsp70+ve cells*

Summary of genetic interaction study

| Genotype | Tumor growth | Hsp70 |
|---|---|---|
| Hsp70$^{RNAi}$ | ↓ | ↓ |
| Hsp70$^{OE}$ | ↓ | ↑ |
| HSF$^{RNAi}$ | Unaltered | Unaltered |
| Sima$^{RNAi}$ | ↑ | ↑ |
| dFOXO$^{RNAi}$ | ↓ | ↓ |
| Bsk$^{DN}$ | ↓ | ↓ |
| Nox $^{RNAi}$ | ↓ | ↓ |

## FINDINGS

- **Hsp70 has a crucial temporal and spacial expression in tumors, which is tightly regulated by tumor micro-environment for its growth. Early and ubiquitous expression in tumor cells, results in tumor regression.**
- **Hippo pathway driven tumors are dependent on ROS mediated JNK and dFOXO signalling axis for Hsp70 expression, bypassing HSF dependent Hsp70 expression. This is first direct report of establishing the role of dFoxo in promoting tumorigenesis.**

## NEXT STEPS

- **To study role of Hsp70 in cellular processes which regulates cytoskeleton arrangements**
- **To understand the relationship between Hypoxia and ROS activity with respect to Sima**

## AKNOWLEDGEMENTS

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