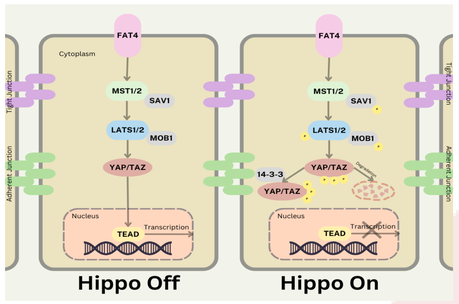
Detection and Relative Quantitation of Changes in Gene Expression of HIPPO Proteins in Doxorubicin-Exposed Human Cells by RT-qPCR

Lillian Fleisher, PharmD Candidate[1], Tricia Domingo, PharmD Candidate[1],
Christopher So, Ph.D. [1], H Stephen Lee, PharmD[1,2], MSc*

1. College of Pharmacy, Roseman University of Health Sciences, Henderson, NV
2. Irma Lerma Rangel School of Pharmacy, Texas A&M Health Science Center, Texas A&M University, College Station, TX

Introduction

- Heat shock protein 27 (HSP27) regulates cellular processes in response to stress, including the presence of chemotherapy drugs.[1] HSP27 affects the HIPPO pathway by suppressing the pathway's phosphorylation cascade.[1]
- Malfunction of this cascade has been linked to the development of cancer and chemotherapy resistance,[1,2] as the HIPPO pathway plays a key role in cell proliferation and tumor suppression. It consists of serine/threonine kinase cascades including MST1/2, LATS1/2 and YAP and TAZ.[3]



- FAT4 is a transmembrane protein receptor that plays a role in the regulation of YAP and possibly the HIPPO signaling pathway.[4]
- In our group's previous work HSP27 mRNA was downregulated by the chemotherapy drug doxorubicin which could also disrupt the HIPPO signaling.

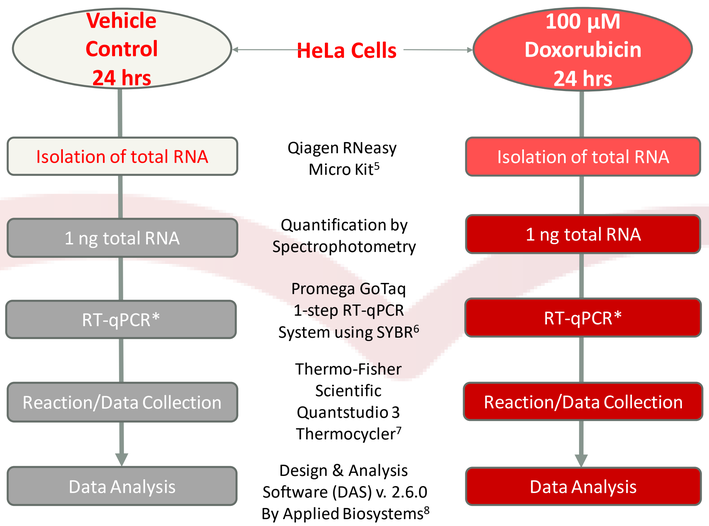
Purpose and Hypothesis

- To detect and quantify the relative changes in gene expression of HIPPO pathway members with the chemotherapeutic drug doxorubicin using an *in vitro* cancer cell culture.
- FAT4 and HIPPO pathway members mRNA expression are hypothesized to increase in drug treated samples compared to controlled samples.

This research has been supported by a 2022-2023 RUHS College of Pharmacy Intramural Research Grant Award

Method

- *In vitro* Hela cells in cell culture were selected as a model and were maintained under standard conditions
- Procedures



Target Genes	Forward PCR Primers	Reverse PCR Primers	Ref.
GAPDH**	AATGCATCCTGCACCACCAA	GTAGCCATATTCATTGTCATA	9
FAT4	CAATAGCTCAGATCCTTTCCT	AACAGTGGCAAAGCTACACCT	10
MST 1	GAACACAGACCTGTGGATTG	CGCCTTGATATCTCGGTGTA	10
YAP	CCCCAAAACGGACAAAGAG	TAGTATCACCTGTATCCATCTC	10
TAZ	GGTGCTACAGTGTCCCCACAA	TTTCTCCTGTATCCATCTCATCCA	10

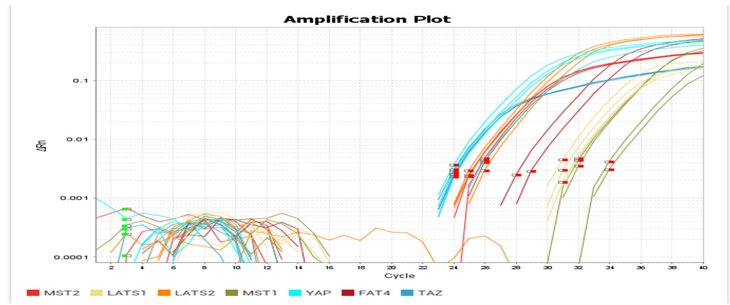
***RT-qPCR reaction conditions: 37°C for 20 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by a melt curve analysis using a total RT-qPCR reaction volume of 20 µL[6,7]**
**** Internal control**

Acknowledgements

- **Dr. David Rawlins and the College of Pharmacy for supporting this project**

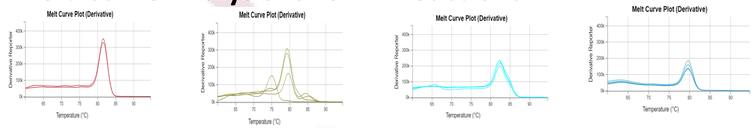
Results

- **Quality of RNA: A260/A280 ratio >1.8 in all samples**



- MST2 and LATS1/2 mRNA were not detected.

Melt-Curve Analysis for SYBR reactions



FAT4 MST1 YAP TAZ

Relative mRNA Quantitation between doxorubicin- and vehicle-treated cells (using GAPDH as internal control), Average RQ ± RQ SEM, n=3, duplicates

FAT4	MST1	YAP	TAZ
177.50% ± 0%	120% ± 29%	80.20% ± 24%	88.55% ± 51%

Discussion and Conclusion

- Not all HIPPO pathway members' mRNA were detected with our methods.
- mRNA expression of FAT4 and MST1 both increased, FAT4 with a variance of 0% and MST1 120% ± 29%.
- The increase in FAT4 mRNA expression in the presence of doxorubicin supports our hypothesis. Increased levels of FAT4 mRNA expression will result in the phosphorylation of YAP and TAZ, ultimately resulting in a decrease in gene expression and cell division.
- To fully comprehend these varying results, further research needs to be completed.

ROSEMAN UNIVERSITY OF HEALTH SCIENCES
TRANSFORMING EDUCATION | REIMAGINING HEALTHCARE | EMBRACING DISCOVERY | COMMITTED TO COMMUNITY

References:

1. Vahid S, Thaper D, Gibson KF, Bishop JL, Zoubeidi A. Molecular chaperone Hsp27 regulates the Hippo tumor suppressor pathway in cancer. *Sci Rep.* 2016 Aug 24;6:31842. doi: 10.1038/srep31842. PMID: 27555231; PMCID: PMC4995483.
2. Zeng R, Dong J. The Hippo Signaling Pathway in Drug Resistance in Cancer. *Cancers (Basel).* 2021 Jan 16;13(2):318. doi: 10.3390/cancers13020318. PMID: 33467099; PMCID: PMC7830227.
3. Wang M, Dai M, Wang D, Xiong W, Zeng Z, Guo C. The regulatory networks of the Hippo signaling pathway in cancer development. *J Cancer*. 2021;12(20):6216-6230. Published 2021 Aug 28. doi:10.7150/jca.62402
4. Che X, Jian F, Jia N, Zheng Y, Jiang Y, Feng W. FAT4-USP51 complex regulates the proliferation and invasion of endometrial cancer via Hippo pathway. *Am J Transl Res*. 2019;11(5):2784-2800. Published 2019 May 15.