The relation between YAP/TAZ expression and cell proliferation in H295RM cells in determining the driving force in L205R caused Adrenal Cushing's Syndrome

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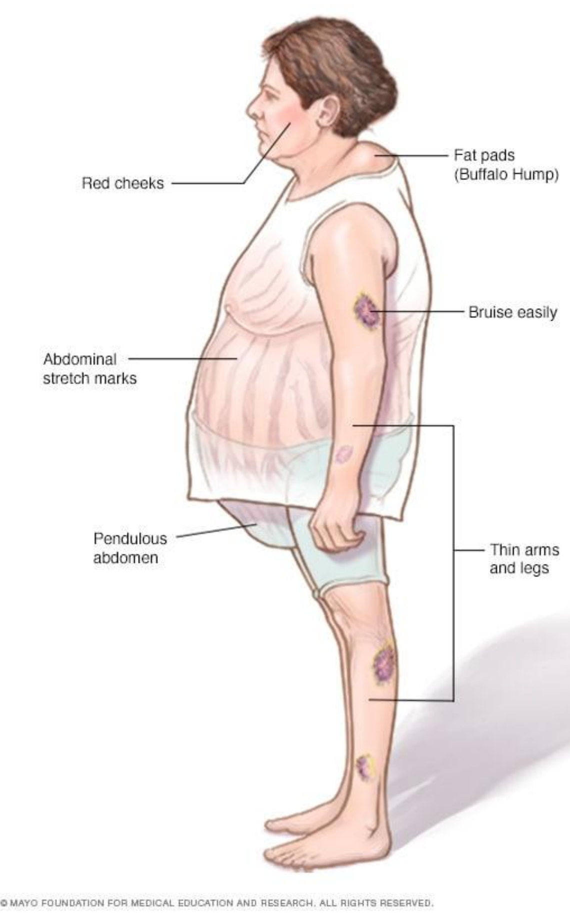
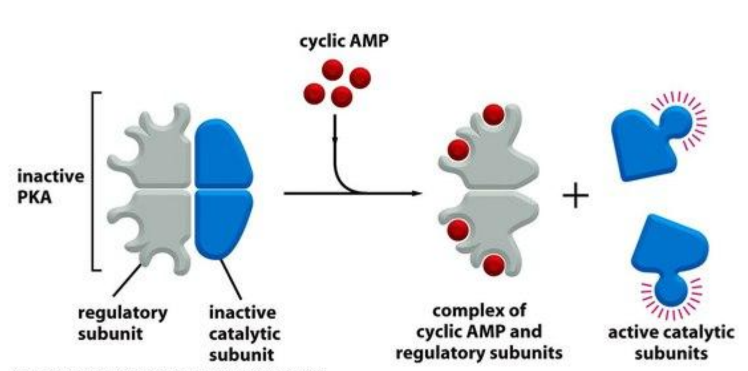
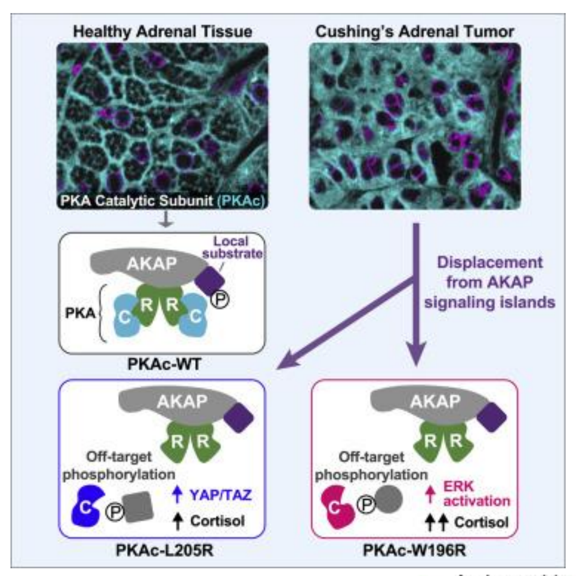
Introduction

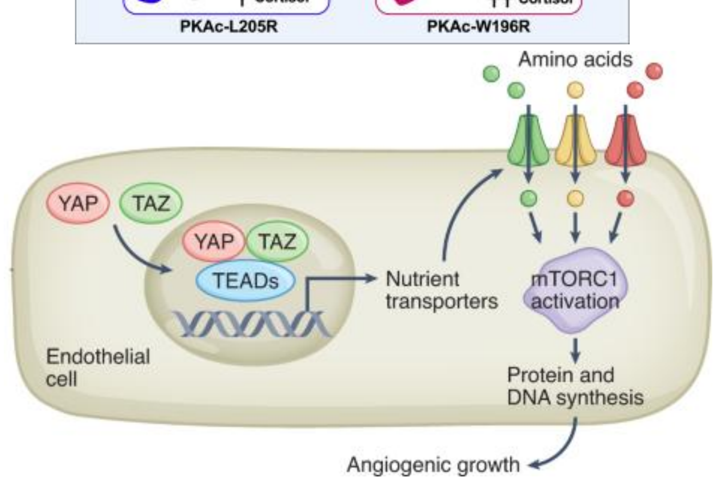
Cushing's Syndrome is a rare disease that affects about 40-70 people out of every million (Steffensen et al, 2010). Cushing's Syndrome causes weight gain, weak muscles, easy bruising, thin arms and legs, a round face, increased fat around the base of the neck and between the shoulders, and wide purple stretch marks significantly decreasing the quality of life of individuals with Cushing's syndrome (U.S. Department of Health and Human Services, 2018). Cushing's Syndrome can even be fatal as it can lead to heart attack, stroke, bone loss, diabetes, and depression (U.S. Department of Health and Human Services, 2018). Cushing's syndrome is characterized by an over production of the "stress hormone" cortisol by the adrenal glands, commonly because of benign adrenal tumors that have a mutation in the catalytic subunit of Protein Kinase A(PKAc). PKAc subunit can no longer bind to the regulatory subunit of PKA; this regulatory subunit forms a dimer that binds to AKAPs. The importance of this interaction is that AKAPs localize PKAc, and mislocalization of PKAc by lack of AKAP incorporation is the driving force is the overproduction of cortisol (Omar et al, 2022).

The PKAc mutant L205R is the most common Cushing's Syndrome mutant, and its off-target phosphorylation causes not only an upregulation of cortisol but also the YAP/TAZ signaling pathway. The YAP/TAZ signaling pathway regulates organ growth and tumorigenesis. YAP/TAZ induces oncogenic growth by activating target genes involved in the control of S-phase entry and mitosis. The goal of this project is to analyze the relationship between YAP/TAZ and cortisol productionThe point of the project is that by better understanding the causes of overproduction of cortisol in Cushing's Syndrome, it will become easier to find better treatments and possibly even cures for this disease.









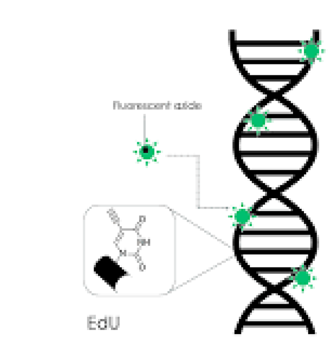
Omar, M. H., Byrne, D. P., Jones, K. N., Lakey, T. M., Collins, K. B., Lee, K.-S., Daly, L. A., Forbush, K. A., Lau, H.-T., Golkowski, M., McKnight, G. S., Breault, D. T., Lefrançois-Martinez, A.-M., Martinez, A., Eyers, C. E., Baird, G. S., Ong, S.-E., Smith, F. D., Eyers, P. A., & Scott, J. D. (2022). Mislocalization of protein kinase a drives pathology in Cushing's syndrome. *Cell Reports*, *40*(2), 111073. https://doi.org/10.1016/j.celrep.2022.111073

Steffensen, C., Bak, A. M., Zøylner Rubeck, K., & Jørgensen, J. O. (2010). Epidemiology of Cushing's syndrome. *Neuroendocrinology*, *92*(Suppl. 1), 1–5. https://doi.org/10.1159/000314297

U.S. Department of Health and Human Services. (2018, May). *Cushing's syndrome - NIDDK*. National Institute of Diabetes and Digestive and Kidney Diseases. https://www.niddk.nih.gov/health-information/endocrine-diseases/cushings-syndrome

Methods

A



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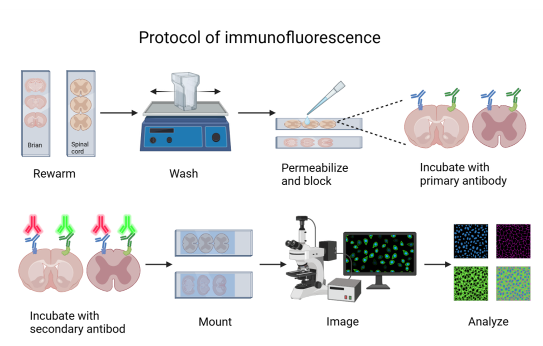

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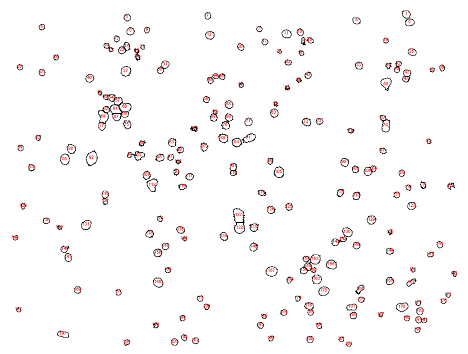

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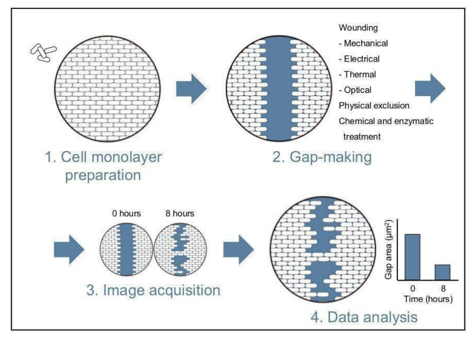

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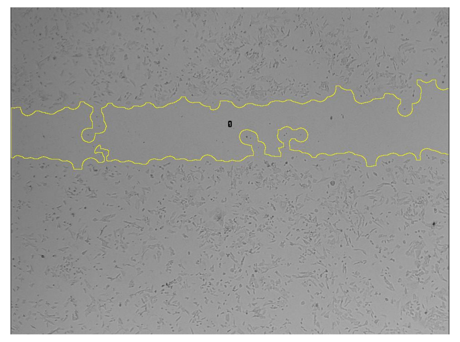


Fig 1. A. 2-hour incubation with thymine analog Edu followed by Click-it reaction with Alexa Flour 647 for staining

B. Immunofluorescence using Keyence with Alexa Flour 647 and Hoechst 33342 staining at emissions of 650/670 nm and 350/461 nm respectively. Alexa Flour 647 targeting Edu and Hoechst 33342 targeting nuclear DNA.

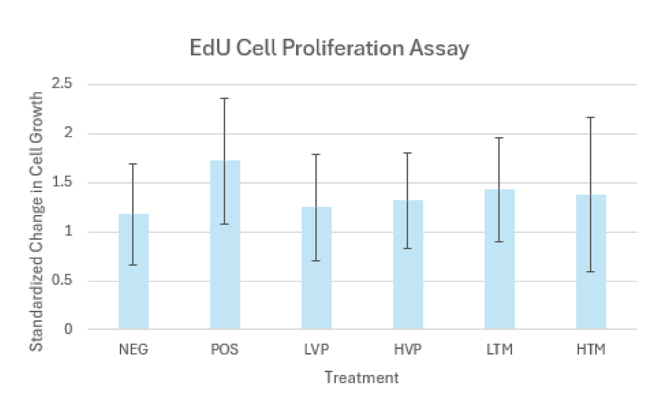
C. Automatic cell counting using ImageJ/Fiji supplemented by hand counting stained cells.

D. Wound heal assay, wound created by physical wounding via scratch with 10 µL pipet tip. Post-imaging, cells incubated for 16-hours.

E. Automatic wound area analysis using ImageJ/Fiji plugin

Results

A



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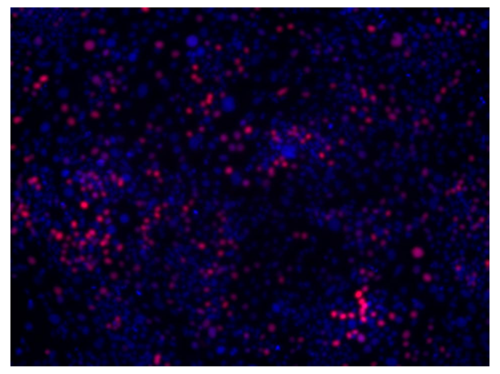
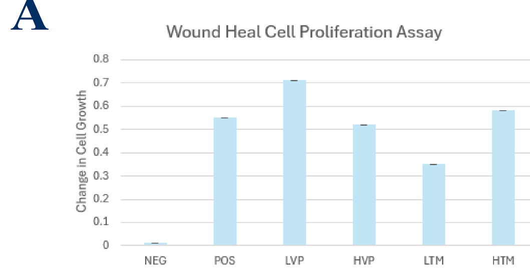


Fig 2. A. No significant difference in change in cell growth from three averaged trials of treatment and the Edu incorporation.

B. Example of cell excitation under immunofluorescence from positive control treatment with 90% H295R media and 10% FBS. Red fluorescence bound to Edu and blue florescence bound to nuclear DNA.

A



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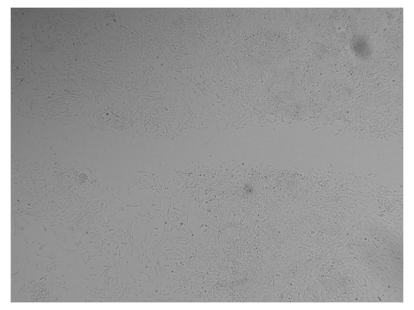

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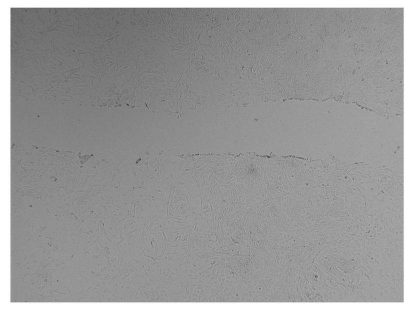

Fig 3. A. All trials showed decreased cell growth compared to control and results were seemingly random.

B. Wound Heal from positive control treatment with 90% H295R and 10% FBS prior to 16h incubation.

C. Wound Heal from positive control treatment with 90% H295R and 10% FBS after 16h incubation.

Conclusions

The current data gathered from this fall of 2024 is inconclusive and points to serious errors in the current treatment conditions. Looking back at the results of the methodology there is some progress that will be productive toward further experimentation. As shown in Fig 2. B and Fig 3. B and C, the Edu and wound heal cell proliferation assays both demonstrated their abilities to analyze the changes in cell growth. This means that going forward, a verified method of determining cell growth can be adapted to find a correlation between cell proliferation and corticosterone levels. There are also some positives as it seems that the positive control is doing a good job in inducing a significant increase in cell proliferation. Further trials will be necessary to reduce any inconsistencies in the protocol, but current results show an almost doubled amount of cell proliferation in the positive treatment group. Overall, the data demonstrates either a deficit in the efficacy, quality, and/or quantity of the treatments given.

Next Steps

Going off the conclusions drawn from the current data, the immediate next step would be a verification of effective drug treatments that both inhibit/induce the YAP/TAZ pathway in such a way that cell proliferation is changing accordingly. More research into the methodology of verteporfin and TM5856 will be conducted, and experiments deducing their quality will also follow. Current plans include western blots and florescent staining to detect if there are noticeable changes in the expression or localization of YAP/TAZ are being made by the current drugs. If not, replacement drug will be purchased, or new treatments will be found.

Once trials achieve the desired results, expanding the research to answer the hypothesis that increased cell proliferation induces elevated cortisol levels in Adrenal Cushing's Syndrome will be conducted. This will be done by conducting an ELISA assay for corticosterone levels following the verified drug treatments from this semester's experiments. Comparing the changes in cell proliferation to changes in corticosterone levels will allow a correlation to be drawn between cell proliferation and cortisol levels.

Trials will also be conducted using genetic alterations to attempt to mirror the disease more closely. Knockdowns of YAP and TAZ plasmids can be used in replacement of verteporfin, and overexpression of YAP and TAZ can replace TM5856 and mirror the results of Cushing's Syndrome. To summarize, the plans are to expand the research to find data on corticosterone levels to finalize the correlation between cell growth and elevated cortisol.