Aditi Subramanyam

Identifying the Role of TEAD Proteins and the Pharmacological Disruption of YAP1 to Inhibit the Function of Oncogenic YAP1 Fusion Proteins

14 million people are diagnosed with cancer globally every year. Cancer is a disease caused by abnormal and rapid cell division, and has the potential to spread to different areas of the body if malignant. There are multiple types of cancer and it is one of the top 10 leading causes of death in the world. Cancer gene fusions are chromosomal aberrations that are thought to have oncogenic function. They are a type of mutation that can be caused by either deletion or translocation in the coding sequence of two genes. The structural rearrangement gives rise to a fusion gene with new properties that are oncogenic in nature, and are driven by the gene that was rearranged. 20% of global cancer morbidity rates are caused by cancer gene fusions. This project looks at a specific subset of cancer gene fusions called YAP1 fusions(fusions driven by aberrations in the YAP1 gene), specifically YAP1-MAMLD1(which is a fusion found in brain tumors) and YAP1-TFE3(which is a fusion found in soft tissue sarcoma). YAP1 (YES Associated Protein 1) is a transcriptional coactivator that drives cell growth ad proliferation and is a proto-oncogene. It has been found to aid metastasis and to be responsible for resisting several chemotherapy treatments including paclitaxel and hence it will be beneficial to look into YAP1 biology for an effective treatment for cancers. Wild type YAP1 does not directly bind to DNA, but needs to interact with other transcription factors, most importantly with TEAD transcription factors to exert YAP1-dependent gene expression and consequently anchorage independent growth. TEAD is a family of 4 transcriptional activator proteins that are necessary to promote several downstream pathways of the YAP/TEAD complex that promote proliferation and metastasis. Some downstream effectors of this interaction include CTGF (connective tissue growth factor), Cyr61(cysteine rich angiogenic inducer), and ANKRD1(ankyrin repeat domain), all of which are proto-oncogenes. It has been found that the TEAD binding domain in the n-terminus of YAP1 is the only conserved domain across all known YAP1 gene fusions, so this project aims to identify the role of each of the 4 TEAD proteins on YAP1 activity in the fusions, in order to test the therapeutic potential of the interactions. The pharmacological efficacy of the drug CA3 was also tested, which is a small molecule that has been found to inhibit YAP1 function through an unknown mechanism that's result is to stop the interaction between YAP1 and TEAD, whose use on the fusion can determine its effectiveness as a treatment route. The project also aims to identify to test the pharmacological efficacy of Dasatinib, a drug that works via triggering nuclear exclusion, which is another method of disrupting the fusion, so testing another potential path in treatment.

I hypothesized that since the TEAD domain was the only domain conserved across all the YAP1 fusions, targeting the interaction between YAP1 and TEAD had the highest potential of being the most effective therapeutic target for these fusions and figuring out which TEAD proteins each fusion relied on would contribute towards creating a more successful treatment.

In order to test the hypothesize, the experiment was split into 3 parts. The first section measured TEAD1-4 expression in the HEK293 cell line (an embryonic kidney cell line) by culturing the cells at different densities, lysing and purifying the RNA, generating cDNA, then using qrt-PCR to measure the gene expression with Sybr Green. The second section assessed which TEAD transcription factors were necessary for YAP1 activity in the wild type and fusions, through transfecting the cells with wild type YAP1, the fusions, and the loading control and reporter plasmid for a luciferase assay. After incubation, the assay was performed. The third part of the experiment was determining the ability of Dasatinib and CA3 to inhibit YAP1 activity in wild type YAP1, which was done by seeding the cells with varying concentrations of each inhibitor, running each through a luciferase assay.

Aditi Subramanyam

Results:

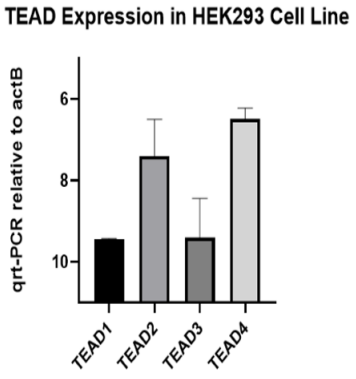

Fig. 1

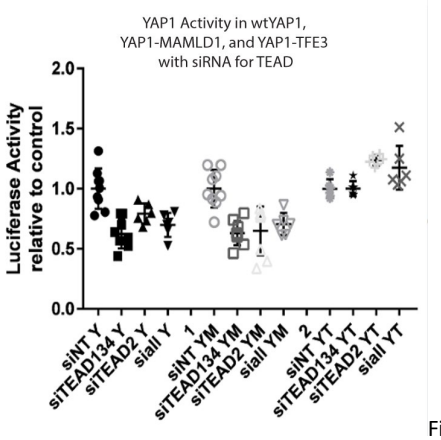

Fig. 2

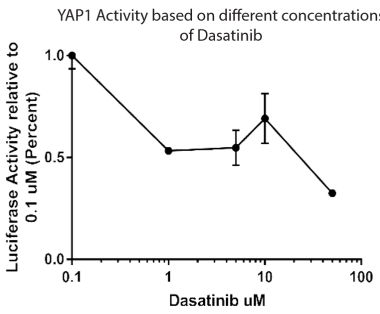

Fig. 3

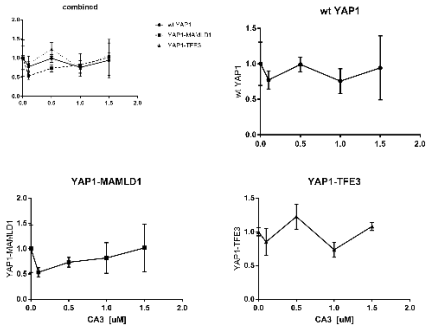

Fig. 4

The presence of all 4 TEAD proteins were determined in the cell line. After graphing the results, it's clear that TEAD2 and TEAD4 are expressed at significantly higher levels than TEAD1 and TEAD3. The average delta ct(difference between cycles of the gene and control) for TEAD2 and TEAD4 are 7.404 and 6.488 respectively, while TEAD1 and TEAD3 had average delta ct's of 9.44 and 9.41, with TEAD2 and 4 having lower delta ct's, which is higher expression(Fig. 1). In the second section of the experiment, wild type YAP1 activity decreased significantly after TEAD134, TEAD2, and TEAD1-4 were silenced with an average negative control value of 0.998 that decreased to 0.564(siTEAD134), 0.95(siTEAD2), and 0.56(siallTEAD). With YAP1-MAMLD1, YAP1 activity also significantly decreased from an average negative control of 0.997 to 0.58(siTEAD134), 0.78(siTEAD2), and 0.706(siallTEAD). However, in YAP1-TFE3, there seemed to be no significant impact by silencing the TEAD protein as the negative control value of 1, stayed the same with siTEAD134, went to 1.22 with siTEAD2, and was 1.17 with siallTEAD(Fig. 2). In the third part of the experiment, in terms of the Dasatinib, it caused a significant impact on YAP1 activity, decreasing it by over 50% from the starting to final concentration(Fig. 3). With the CA3, it surprisingly didn't have a significant impact on YAP1 activity, not changing overall in either wild type or the fusions(Fig. 4).

The experiment aimed to identify the role of the 4 TEAD proteins in YAP1 fusions as well as look at the efficacy of the drug Dasatinib which works via triggering nuclear exclusion as another approach to the YAP1 fusions. The project confirmed the presence of all 4 TEAD proteins in the cell line. Using One Way Anova, it was determined that TEAD2 and TEAD4 were expressed at significantly higher levels compared to TEAD1 and 3(padj. < 0.002). Subsequently, wild type YAP1 (One-Way ANOVA padj.=0.0004) and YAP1-MAMLD1(padj.<0.0001) showed a significant reduction in YAP1 activity upon silencing of TEAD1-4 expression while that silencing seemed to have no significant effect on YAP1-TFE3(padj.=0.53). Lastly, Dasatinib treatment lead to a significant reduction of wild type YAP1 activity(p=0.002), yet CA3 seemed to have no effect on wild type YAP1 or either of the fusions(p=0.03). The

results of the experiment have determined that YAP1 activity in the fusions have major reliance on binding with the TEAD protein.

In the future, testing Dasatinib on the fusions will be performed as well as investigating the reliance of the YAP1 fusions on each TEAD protein specifically, as due to time constraints, siRNA;s for all TEAD proteins weren't able to undergo testing, as well as using a variety of drugs to look at pharmacological disruption of the YAP1 activity. The project also hopes to expand to looking at different downstream effectors of the YAP1 pathway, and the potential of downstream signaling pathways of YAP1 as effective therapeutic targets. But for looking at the very first YAP1 interaction, the experiment has determined that the YAP1/TEAD complex has a high potential for being a therapeutic target for stopping cancer proliferation and metastasis. The surprising result comes from the negligible effect of CA3 on the fusions, which could be due to the fact that the mechanism of how the molecule inhibits YAP1/TEAD binding remains unknown, meaning it could rely on other factors to exert its effects on their binding, and these factors may not be present in the fusion, which would require further investigation. Another surprising result was that of the reliance of TFE3 on the fusions, which seem to be none, though the fusions have the TEAD interaction domain. In the future, conducting a co-immunoprecipitation test on the fusions would be a useful tool in determining the binding properties between YAP1 and TEAD in the fusions and explain the results seen from these experiments. YAP1 is already overexpressed in several cancer types and associated with a poor survival rate, so further investigating its biology and interactions would be beneficial.

Citations:

1. Deel MD, Li JJ, Crose LE, Linardic CM. A Review: Molecular Aberrations within Hippo Signaling in Bone and Soft-Tissue Sarcomas. Front Oncol. 2015;5: 190.
2. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. Cancer Cell. 2015;27: 728-743.
3. Antonescu CR, Le Loarer F, Mosquera JM, Sboner A, Zhang L, Chen CL, et al. Novel YAP1-TFE3 fusion defines a distinct subset of epithelioid hemangioendothelioma. Genes Chromosomes Cancer. 2013;52: 775-784.
4. http://54.84.12.177/PanCanFusV2/.

5. Zhao, B., Kim, J., & Guan, X. Y. (2009, February 01). Both TEAD-Binding and WW Domains Are Required for the Growth Stimulation and Oncogenic Transformation Activity of Yes-Associated Protein. Retrieved from http://cancerres.aacrjournals.org/content/69/3/1089.long

6. Defining the Molecular Landscape of Ependymomas. (2015, May 11). Retrieved from https://www.sciencedirect.com/science/article/pii/S1535610815001488

7. Mitelman, F., Johansson, B., & Mertens, F. (2007, April). The impact of translocations and gene fusions on cancer causation. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17361217