Aspects Affecting the Expression of Hypoxia-Inducible Factors in Cardiomyocytes

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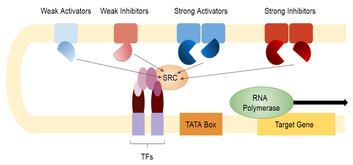
ABSTRACT

By comparing the immunofluorescent images of each of the wells containing cells in different conditions, we can compare signals that are relevant to diabetic cardiomyopathy change HIF expression, activity, or localization. The cells were put in normal media, $CoCl_2$, or isoproterenol, and were transfected with HA HIF-1α and HA HIF-2α. We added only the 2nd antibody to one of the wells for a negative control. The signals from this well would show how much of the other signals should be expected as background. We knew from prior research that $CoCl_2$ should increase HIF expression and used cells in $CoCl_2$ as a positive control. The mouse HIF-1α antibody (ab113642) can be detected using cyanine 5 dye (CY5) because it emits a wavelength of 488. Similarly, the rabbit HIF-2α antibody (PA1-16510) can be detected using green fluorescent protein (GFP) due to its wavelength of 647. The results supported previous studies from other cell types. The cells in $CoCl_2$ were transfected with HIF-1α and HIF-2α overexpressed the HIFs in comparison to those in normal media. Additionally, we found that cells treated to induce hypertrophy also may increase HIF expression.

Background

Diabetes is one of the leading causes of death in the United States due to its comorbidities. One of which is an increased likelihood of heart failure. Research has shown that a diabetic environment diminishes the stability and functionality of HIF-1α.

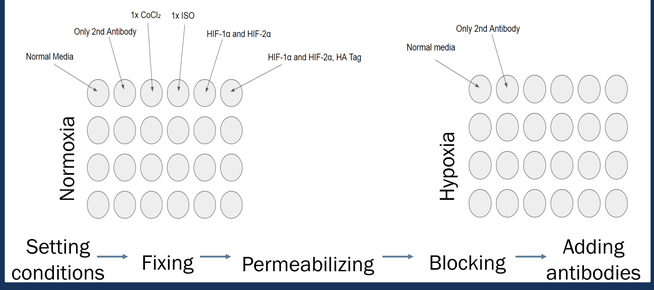
Hypoxia inducible factors (HIFs) are transcriptional factors that help in regulating a cellular response when oxygen levels are low, yet there is evidence that there is very little redundancy between HIF-1α and HIF-2α. HIF-1α mostly regulates metabolic and early angiogenic transcriptional targets whereas HIF-2α manages later angiogenesis and vessel maturation. HIFs are made in normoxic conditions too; however, they are rapidly degraded via the ubiquitin-proteasome pathway.



Materials

H9C2 cells and 293-HEK cells, Dulbecco's Modification of Eagle's Medium 1X with 1 g/L glucose, L-glutamine and sodium pyruvate, eight 24-well plates, Dulbecco's phosphate buffered saline (DPBS), $CoCl_2$, isoproterenol (ISO), 1X, fixing solution zinc formaldehyde fixatives, permeability solution (PBS and 0.2% Tritonx100), blocking solution 1% BSA in TBST, and 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride, 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) fluorescent dye, antibodies HIF-1α ab113642, HIF-2α PA1-16510, HIF-2α NB100-122, mutated HIF-1 α MA1-16519, and mutated HIF-2α NB100-105

Methods



Result

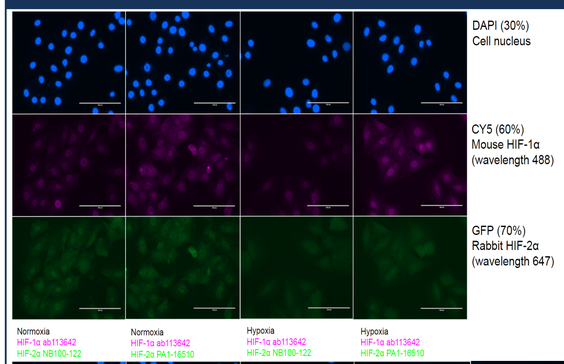
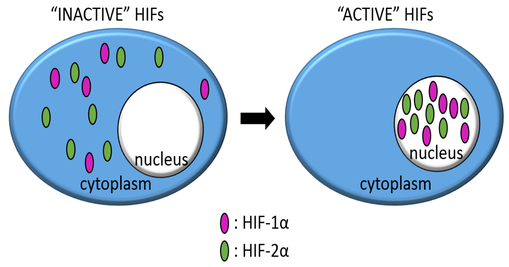


Figure 1



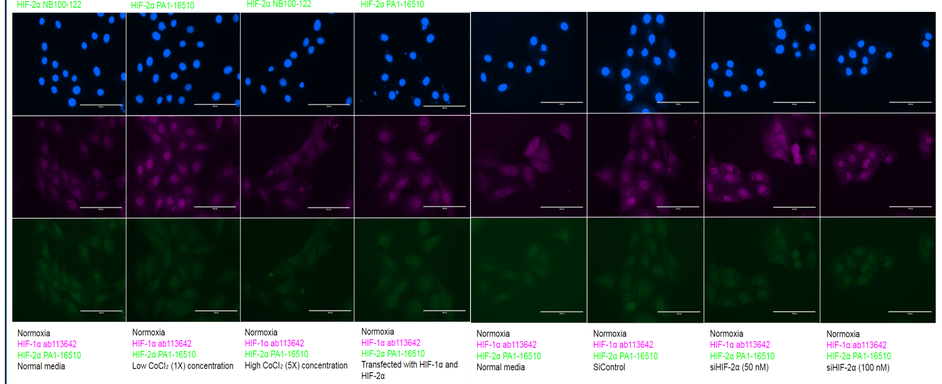


Figure 2

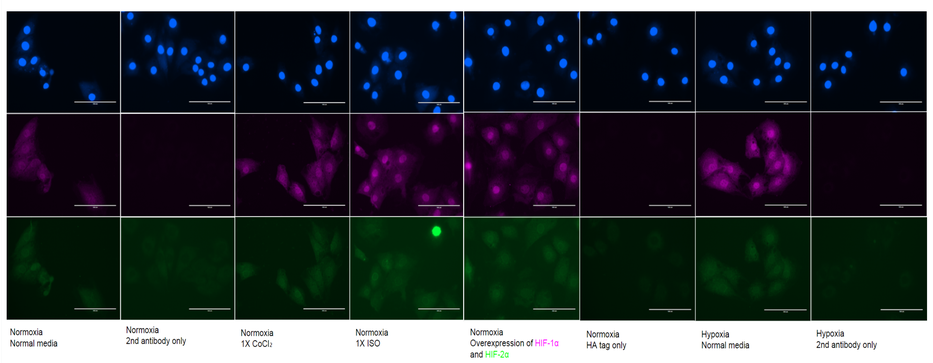


Figure 3

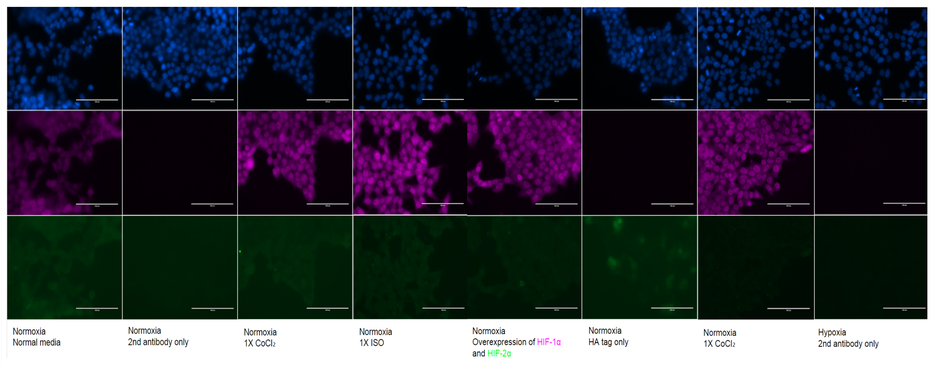


Figure 4

Discussion

Figure 1: Based on these two combinations of antibodies in normoxic and hypoxic conditions, the combination of HIF-1α ab113642 and HIF-2α PA1-16510 should be used because HIF expression was more noticeable than in the cells treated with NB100-122. Although the cells in hypoxia should have more HIF expression than those in normoxia, we think that we fixed the cells too slowly which might have caused the HIFs to be degraded before the pictures were taken. In fact, studies have shown that approximately 50% of HIFs may be degraded within five minutes.

Figure 2: For the cells in the 1X $CoCl_2$ concentration, there is more HIF expression than for those in the higher 5X concentration. According to previous studies, $CoCl_2$ should have increased the expression of HIFs. However, for cells in a high concentration of $CoCl_2$ concentration, the cells could have been damaged due to the high concentration. Therefore, for follow-up experiments, we decided to use the 1X concentration rather than the 5X to get optimal HIF expression.

Figure 3: There is no significant reduction in the signaling of HIF-1α or HIF-2α. However, there is a slight increase in the expression of HIF-1α. Overall, the knockdown of HIF-1α and HIF-2α failed, so we decided to repeat the experiment with not only the H9C2 cell line but also the 293-HEK cell line which is known to have a high transfection rate. The cells in $CoCl_2$ and ISO had more expression than those in normal media.

Figure 4: Although the signals were more apparent in the 293-HEK cells than the H9C2 cells, the cells were more crowded as well. However, this arrangement is because of human error. I, while washing the cells, unknowingly pushed the cells towards the edges of the well because the 293-HEK cells do not adhere very strongly to the plate. The signaling of the HIF-1α in 293-HEK cells reflects previous research findings better than the H9C2 cells. For example, compared to the cells in normoxia, those in CoCl and ISO have a significant increase. However, the HIF-2α has such a weak signal that we cannot distinguish any changes in expression from cells in normoxia to cells in other conditions.

Conclusion and Future Endeavors

- The HIF-2α had weak signal in the human 293-HEK cells.
- Overall, the efficiency of transfection in H9C2 was low.
- $CoCl_2$ stabilized HIF expression.
- ISO may increase the expression of HIF-1α.
- These results helped us decide what reagents and cells we should use in later experimentation to study HIF-1α and HIF-2α targets in cardiomyocytes.

Acknowledgments

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