The Effects of Hypoxia on Chromatin in Cardiomyocytes

Abstract

Heart disease constitutes one out of four deaths in the United States. During a myocardial infarction (MI), commonly known as a heart attack, heart muscle cells (i.e., cardiomyocytes) are exposed to a low oxygen environment (i.e., hypoxia). Due to hypoxia, cell death occurs with subsequent physical remodeling of the heart. The left ventricular chamber enlarges to maintain cardiac output, and becomes more spherical, which can impair the efficiency of contraction. Chamber enlargement increases wall stress which increases oxygen demand by the myocardium. This can result in hypoxia in the myocardium and stabilization of Hypoxia Inducible Factor 1 (HIF-1). HIF-1 plays a crucial role in gene regulation during hypoxia. Genes involved in processes such as angiogenesis, the production of new blood vessels, and anaerobic glycolysis, an energy producing pathway that is dominantly expressed when oxygen availability is low, are upregulated by HIF. Gene regulation is dependent on the state of chromatin, the organized structure of DNA wrapped around histones. Open regions of the genome, where genes are available to the transcriptional apparatus, are designated as euchromatin, and closed regions where chromatin is tightly packed and genes are not accessible, as heterochromatin. Chromatin can be modified by histone modifications that are associated with specific transcriptional states. Specifically, H3K27me3 and H3K4me3 histone modifications have been shown to be associated with decreased and increased gene activity, respectively. This study seeks to understand the effects of hypoxia on the dynamics of these two histone modifications marks within cardiomyocytes. We plan to conduct a genomic-wide analysis of histone modifications in cardiomyocytes to obtain a deeper understanding of heart-specific gene regulation during hypoxia.
Hypothesis

It is hypothesized that histone modification levels will increase in hypoxic cardiomyocytes in comparison to normoxic cardiomyocytes and that this increase will be dependent of HIF.

Specific Aims

We will test this hypothesis with three specific aims. In aim 1, the effect of hypoxia on the total levels of H3K4me3 and H3K27me3 will be determined in mouse cardiomyocytes. In aim 2, the role of HIF-1 in these changes will be assessed by utilizing a cardiomyocyte-specific conditional knockout of HIF1α. In aim 3, changes in the distribution of H3K27me3 and H3K4me3 across the genomes of cardiomyocytes resulting from hypoxia will be measured.

Significance

The heart is dependent on a reliable supply of oxygen for the very high levels of ATP synthesis that it requires for constant contractile work. However, little is known regarding the genome-wide changes to chromatin in response to hypoxic conditions in cardiomyocytes. Ischemic heart disease can lead to hypoxia and subsequent changes in gene expression. This study will contribute to the deeper understanding of the hypoxic response within the heart on a molecular level and may reveal potential therapeutic targets for treating ischemia.
Background Information and Literature Review

Histone modifications during hypoxia

Under low-oxygen conditions, the Hypoxia Inducible Factor (HIF) pathway is activated within cells, ultimately leading to the upregulation of many HIF-responsive genes. For example, vascular endothelial growth factor A (VEGFA), a gene responsible for promoting the growth of new blood vessels, is regulated by HIF-1 during hypoxia. HIF-1 is comprised of two subunits, HIF-1α and HIF-1β. In the presence of oxygen, Prolyl Hydroxylase Domain (PHD) enzymes and Von-Hippel Lindeau (VHL) factor work together to ubiquitinate HIF-1α, leading to degradation by proteasomes. In the absence of oxygen or very low oxygen levels during hypoxia, however, HIF-1α stabilizes and enters the nucleus, dimerizes with HIF-1β, and binds to Hypoxia Response Elements (HREs) in the promoter regions of HIF-responsive genes. This leads to the activation of many genes such as VEGFA and genes associated with the energy-producing pathway known as glycolysis. While the best studied mechanisms of gene regulation in response to oxygen levels involve HIF, other mechanisms of oxygen sensing have been identified. KDM6A, for example, which is a histone demethylase enzyme that removes methyl (CH₃) groups from lysine residues of a histone tail to
regulate gene expression, contains oxygen sensors that allow for the recognition of the amount of oxygen within the tissues and cells.\(^1\)

<table>
<thead>
<tr>
<th>Disease state</th>
<th>Changes to lysine methylation status</th>
<th>Gene locus and change in expression level</th>
<th>KDM implicated?</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Heart failure</td>
<td>H3K4me3</td>
<td>Global study</td>
<td>-</td>
<td>[169]</td>
</tr>
<tr>
<td>PTIP knock out mouse (reduced global H3K4me3 model)</td>
<td>H3K4me3 ↓ (globally induced)</td>
<td>KCNIP2 ↓</td>
<td>-</td>
<td>[170]</td>
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<tr>
<td>Cardiac hypertrophy</td>
<td>H3K9me3 ↓</td>
<td>FHL1 ↑</td>
<td>KDM4A ↑</td>
<td>[171]</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>H3K36me3 ↓</td>
<td>DUX4 ↓</td>
<td>-</td>
<td>[172]</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>H3K9me2/3 ↓</td>
<td>ANP ↑</td>
<td>KDM3A ↑</td>
<td>[173]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BNP ↑</td>
<td>KDM4A ↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>KDM4B ↑</td>
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</table>

Several studies have demonstrated changes in gene expression levels and associated changes to histone lysine methylation in cardiovascular disease states. Altered KDM expression levels were also reported in some cases.

**Figure 2.** A table provided by Hancock et al. that depicts the different diseases and the different changes to gene expression, histone modification levels and KDMs.\(^2\)

There are also enzymes known as Jumonji C lysine demethylase enzymes (JmJC-KDMs) and histone lysine methyltransferases (KMTs) that remove or add methyl groups, respectively, from lysine amino acids on histone tails.\(^2\) Some genes, such as VEGFA, that are regulated during hypoxia have been identified. However, there is still not much known about gene regulation or the specific genes that are regulated during hypoxia within cardiomyocytes. There are currently 21 KDM enzymes that have been identified and some of these KDMs have been implied to be upregulated in certain heart diseases such as cardiac hypertrophy and cardiomyopathy.\(^2\) There are also 33 lysine methyltransferases (KMTs) that have been currently identified. As this project focuses on two histone modifications marks, H3K27me3 and H3K4me3, it is also important to recognize that not all KDMs or KMTs target either or both of these modifications. Figure 2 depicts different heart disease states that result in the changes in histone modification levels, gene expression, and levels of KDMs.\(^2\)
Most notably is how there are three KDMs being upregulated due to cardiomyopathy, which is also accompanied by the increase of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). The increase in KDMs has only been implicated, as stated in Figure 2, meaning that more research would have to occur in order to fully understand this upregulation of KDMs. However, what is known is that ADP and BNP are known as fetal genes and Hancock et al. suggested that the upregulation of BNP and ANP may serve as a cardioprotective response.²

While looking at the relationship between cardiovascular disease, hypoxia and the JmjC-KDMs, Hancock et al. also observed a shift in prioritizing gene expression from fatty acid oxidation to glycolysis and the reactivation of fetal genes such as atrial natriuretic peptide and brain natriuretic peptide”.² This is understandable as, due to the low levels of oxygen during hypoxia, glycolysis should be prevalent within hypoxic cells as it is an energy producing pathway that does not require high amounts of oxygen that other energy producing pathways require such as oxidative phosphorylation. ANP is involved in lowering blood pressure and maintaining a balance of electrolytes within the heart while BNP is released during times of volume expansion and increased wall strength within cardiomyocytes. Lowering the gene

![Figure 3. Immunofluorescence provided by Batie et al. of normoxic (top row) and hypoxic (bottom row) HeLa cells with accompanying scatter plot quantification of intensity.³](image-url)
expression of KDM3A and KDM4A resulted in the changes in expression of the previously mentioned ANP and BNP. This shows that abnormal or impaired regulations of KDM3A and KDM4A expression levels could provide detrimental effects to the proper functioning of the heart. Among BNP and ANP are most likely other genes that are regulated and affected by the levels of KDMs; however, the identification of many of these genes are still currently being researched.

Levels of certain histone modifications have been observed to increase in hypoxic cells compared to normoxic cells (Figure 3). Specifically, levels of H3K27me3 and H3K4me3 in HeLa cells were shown to accumulate in hypoxic HeLa cells compared to normoxic cells. This suggests that the KMTs responsible for catalyzing these marks were being inhibited or the KDMs that remove these marks were being hyperactivated. This identification of the changes in histone modification levels sparked this proposed project to look at the same histone modification marks within cardiomyocytes, since these cells are likely to have a much different response to hypoxia than immortal cell lines such as HeLa cells. Specific genes that accumulated or were depleted of these methyl marks were also identified in HeLa and HFF cells through ChIP-seq and RNA-seq and included genes important for cell proliferation, cell differentiation, and apoptosis or programmed cell death. Utilizing the same technique of ChIP-seq within cardiomyocytes can provide a direct connection of the hypoxic gene regulation response to the heart. It is important to note here that, within the heart, there are many different cell types besides just cardiomyocytes. Fibroblasts, endothelial cells and smooth muscle cells are also cells found within the heart that have different responses to hypoxia in comparison to the response in cardiomyocytes. These cells also have different chromatin landscape and arise from different cell lineages so it would be expected to see differences between the hypoxic response in these cells in
comparison to cardiomyocytes. How chromatin directly responds to oxygen levels through HIF-independent mechanisms is an active area of research and should reveal important insights into transcriptional responses to environmental stimuli.

Another enzyme that regulates H3K4me3 levels during hypoxia is Prolyl Hydroxylase Domain (PHD) finger protein 8 (PHF8). This enzyme was observed to be in increased abundance slightly after 24 hours and due to PHF8, H3K4me3 levels on hypoxia-inducible genes being maintained. To show PHF8 was regulating H3K4me3 levels and that it was not pure coincidence that H3K4me3 increased in the presence of the PHF8 enzyme, PHF8 activity levels were purposefully decreased, which also resulted in the decrease of H3K4me3 levels as well. This decrease of H3K4me3 levels was observed using chromatin immunoprecipitation sequencing in Human Embryonic Kidney cells (HEK293T cells), focusing on specifically the KDM3A, ENO2 and HIF1A genes. The enrichment between PHF8 and H3K4me3 was observed and it was discovered that at all three transcription sites of these three genes, H3K4me3 levels were reduced while the PHF8 gene expression levels were also reduced. This further solidified the evidence that PHF8 regulates H3K4me3 levels.

With demethylases like KDM6A that remove methyl groups from lysines, it also important to mention enzymes that add methyl groups. Specifically for H3K27me3, Polycomb Repressive Complex 2 (PRC2), which contains the EZH2 enzyme, catalyzes the addition of H3K27me3 to target genes. If H3K27me3 levels increase during hypoxia within cardiomyocytes, then a possible explanation for this increase would be due to the upregulation of PRC2.

Chromatin regulation

As histone tails can be modified, DNA itself can also be regulated. One of the main types of DNA modifications is DNA methylation. It is known that DNA methylation are found
typically near CpG islands, which are cytosine and guanine found multiple times in a row on the same side of the DNA molecule. DNA methylation is commonly associated with the suppressing of gene activity, forming heterochromatin. It was discovered that methyl binding domain proteins (MBDs) attract the histone modification mark H3K9me. In the absence of DNA methylation, H3K9me3 levels, the levels of the tri-methylation of the lysine on the 9th position of the Histone 3 tail, decreased within heterochromatic regions of DNA. However, the connection between histone modification marks and DNA methylation was investigated even further. H3K4me3 modification marks were not found by regions with DNA methylation due to the ADD region of DNA methyltransferases, enzymes that add methyl groups to DNA, being blocked from binding to the H3 tail if H3K4 is methylated. On the other hand, H3K27me3 does not have this interaction so H3K27me3 marks can be found by DNA methylation regions and thus are not regulated the same way H3K4me3 is.

Rose and Klose reiterated from other studies that H3K4me3 histone modification marks are commonly associated with gene activation or increases in gene expression. On the other hand, H3K27me3 is commonly associated with gene repression or decreases in gene expression. Both of these claims are supported because H3K4me3 levels are high where there is no DNA methylation that represses gene expression, while H3K27me3 levels can be high in the presence of these highly methylated DNA sequences.

Besides histone methylation modifications such as H3K27me3 and H3K4me3, it is important to also identify that histone acetylation is also present within the cell. Histone acetylation is the addition of acetyl groups (C\(_2\)H\(_3\)O) to histone tail proteins and these modifications are normally associated with high gene expression and euchromatin or open chromatin. Unmethylated DNA was found to be observed wrapped around histones that have
acetylation on their tails, which makes sense as both histone acetylation and demethylation encourages active gene expression.\textsuperscript{6}

As it is known that adult cardiomyocytes have very limited regenerative properties as well as a differentiated state that turns off and inhibit genes that are not important for the functioning of a cardiomyocyte, Cedar and Bergman looked at somatic cell reprogramming to observe any relationships that somatic cell reprogramming has with DNA methylation.\textsuperscript{6} It was observed that somatic cells can become an induced pluripotent cell (iPS cell), which allows the cell to then have the ability to differentiate or become almost any cell type within the body.\textsuperscript{6} This ability is due to the removal of all of the DNA modifications, such as DNA methylation, from the pluripotency genes by specific proteins found within the cell.\textsuperscript{6} In fact, Cedar and Bergman stated that the inhibition of G9a, which is a histone lysine N-methyltransferase, also stimulates the reprogramming of these somatic cells, further supporting the link between DNA methylation and histone methylation.\textsuperscript{6} The application of induced pluripotency and the identification of proteins such as G9a can lead to future research to restore regenerative properties to adult cardiomyocytes other than by increasing the expression of cyclins and cyclin-dependent kinases.
Heart-specific hypoxia response

Finally, this discussion of hypoxia and gene regulation will be tied back to the heart. When exposed to a hypoxic environment, cardiomyocytes are observed to have lower ATP concentrations as well as an increase of glucose transport into the cell and anaerobic glycolysis, which transforms the pyruvate made from glycolysis into lactate and alanine.7 Davies and Wedzicha also stated that the stroke volume, the amount of blood pumped by one contraction of the left ventricle of our heart, gradually decreased after travelling and staying at high altitudes where the oxygen concentration in the air is lower.7 A small increase then decrease to 25% lower coronary blood flow than pre-ascent coronary blood flow was also observed when climbing high altitudes which may result from an increased unloading of oxygen in capillary beds.7 This suggests that less oxygen may be unloaded in the heart, which may also explain the hypoxic cardiomyocyte cell death found during a heart attack due to the lack of oxygen availability.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Perinatal remodeling: a list of the main changes in cardiomyocytes during the transition from the prenatal to the postnatal phenotype.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal heart</td>
<td>Postnatal heart</td>
</tr>
<tr>
<td>Number of nuclei</td>
<td>1 nucleus</td>
</tr>
<tr>
<td>Main source of energy</td>
<td>Glycolysis</td>
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<tr>
<td>Mitochondria</td>
<td>Small, immature</td>
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<tr>
<td>Myofibrils</td>
<td>Poorly organized</td>
</tr>
<tr>
<td></td>
<td>β-myosin heavy chain</td>
</tr>
<tr>
<td></td>
<td>Slow skeletal muscle isoforms</td>
</tr>
<tr>
<td>Adherence junctions</td>
<td>Surround the cells</td>
</tr>
</tbody>
</table>

Figure 4. A table provided by Cerychova and Pavlinkova that highlights the main changes that occur between cardiomyocytes in the prenatal heart and postnatal heart.8
With the fetus developing within the placenta of a mother, this fetus is being exposed to a hypoxic environment. Researchers can then safely assume that HIF-1 is important in heart development, cardiac metabolism remodeling and adult heart functions. Deleting the *Hif1α* gene from every cell within the body resulted in the death of the embryo by the tenth day after seeing the vaginal plug on a pregnant mouse (i.e. E10). This death was due to heart defects such as the “improper formation of the heart tube...to the defective looping of the heart tube.” After birth, cardiomyocytes switch metabolism pathways from the use of glucose in glycolysis to the oxidation of fatty acids in β-oxidation. This switch is not completely understood but there is data that suggests that this switch may be the result of fatty acid availability from breast milk as well as the downregulation of the *Hif1α* gene due to the neonatal mouse no longer being in a hypoxic environment. Since individuals are no longer in a hypoxic environment after birth, a switch from majorly using anaerobic glycolysis to oxidative phosphorylation and β-oxidation, which utilizes the mitochondria in cells, is done to provide an ample amount of energy in the form of ATP.

Figure 4 depicts the main changes that occur between cardiomyocytes within a prenatal heart and cardiomyocytes within a postnatal heart. Most notably, the presence of more than one nucleus shows that there are now at maximum four copies of the genome that can be accessed, leading to the increased levels of gene expression/inhibition as well as the increased/decreased levels of histone modifications. The observed increase in mitochondria between prenatal and postnatal cardiomyocytes also is reasonable as once the mice or offspring are born, they are no longer in a hypoxic environment so they can utilize more mitochondria to produce energy whereas anaerobic glycolysis is a process that does not use the mitochondria.
With this introduction to normal oxygen levels, i.e., normoxia, cardiomyocytes should take advantage of the highly efficient energy production pathway of oxidative phosphorylation, rather than relying on anaerobic glycolysis, which produces less energy. To further support this point, when HIF-1 is overexpressed within the heart, glucose utilization through anaerobic glycolysis increases. Pyruvate dehydrogenase kinase is induced/upregulated during times where HIF-1α is expressed in high quantities to prevent pyruvate from being converted into Acetyl-CoA, a substrate necessary for cellular respiration. This induction is necessary because, as mentioned earlier, during hypoxia where there is low amounts of oxygen, cellular respiration is not optimal. Instead, cells such as cardiomyocytes rely heavily on anaerobic glycolysis to provide the necessary energy to survive. Since HIF-1 is no longer necessary at all times due to the normoxic environment of Earth, however, oxidative phosphorylation’s dominance in the production of ATP for the cell is in part due to the decrease in HIF-1α availability within tissue.

What is also important to note is the protective features that HIF-1 expression has within the heart. Wu et al. observed that hearts expressing HIF-1α tolerated ischemia, which is reduced oxygen levels within the heart due to a lower amount of blood flow, better than hearts that did not express HIF-1α. Hearts with HIF-1α expression had a higher viable tissue percentage in comparison to hearts without HIF-1α, suggesting that HIF-1α reduces the amount of damage caused by ischemia. ATP levels were also maintained more sufficiently in hearts that express HIF-1α. This would allow the heart to continue metabolic pathways that require energy in the form of ATP.
Methodology/Research Design

Data Collection

To fulfill aim 1 and observe levels in histone modification levels of both hypoxic and normoxic cardiomyocytes, cardiomyocytes will first be isolated from the other cells within the heart, such as fibroblasts and endothelial cells. These cardiomyocytes will then be placed into one of two conditions; cardiomyocytes will either be exposed to a hypoxic environment of 1% oxygen with the use of a Baker Ruskin Invivo2 400 hypoxia machine or the cells will be placed in a 37 °C incubator, which will provide a normoxic environment or normal oxygen level of 21% oxygen. To then measure these levels of histone modifications within the neonatal cardiomyocytes, the technique of immunofluorescence will be utilized. Immunofluorescence utilizes fluorescent secondary antibodies that binds to primary antibodies that recognize a specific target protein, in this case H3K27me3 or H3K4me3, which are both histone modifications found on the protein tail of histones, to qualitatively, and with further analysis even quantitatively, measure protein levels utilizing a confocal microscope to observe fluorescence. In this case, brighter intensities correlate to higher amounts of the histone modifications. If hypoxia inhibits demethylases like KDM6A, it is expected that histone modification levels increase during hypoxia in comparison to normoxia. In other words, a brighter fluorescence would be observed in cardiomyocytes exposed to hypoxia in comparison to cardiomyocytes exposed to normoxia.

As a complementary experiment to the immunofluorescence staining, Western blotting, which also utilizes specific primary antibodies that recognize either H3K27me3 or H3K4me3, will be used. Similar to what is shown in Figure 5, total lysate extracted from normoxic and
hypoxic cardiomyocytes will be run on a polyacrylamide gel to separate the histone modifications from other proteins found within the cardiomyocyte. These proteins will then be transferred to a blotting sheet made of nitrocellulose. From there, the antibodies that recognize H3K4me3 or H3K27me3 will be added and the blotting sheet can then be imaged. Secondary antibodies that recognize the constant region of the primary antibodies will then be added. Importantly, these secondary antibodies are conjugated to a fluorophore, a fluorescent molecule that allows for the visualization of the target protein, H3K4me3 or H3K27me3 in this case. Western blotting will be used in addition to immunofluorescence to provide quantitative values of protein amount by measuring the signal intensity or brightness of the bands on the blotting sheet after imaging. Within the obtained image, the brighter/darker the band is, the higher amount of the protein of interest is observed. With both immunofluorescence and Western blotting, quantitative and qualitative data will be collected.

To fulfill aim 2, a HIF-conditional knockout mouse strain provided by the Jackson Lab in Maine will be utilized to remove the Hif1α gene from only cardiomyocytes. This specificity of removing HIF only from cardiomyocytes is driven by α-MHC Cre recombinase. α-MHC, which
stands for alpha-myosin heavy chain, is only expressed within cardiomyocytes, which allows for the Hif1α gene to only be removed from cardiomyocytes while leaving the Hif1α gene in every other cell type found within the heart and in the body. The Hif1α gene is flanked on both sides by loxP sites, which will direct α-MHC Cre to target and remove the Hif1α gene. This will then remove the production of HIF within these specific cardiomyocytes. The HIF-conditional knockout mice will be crossed with another mouse strain that contain α-MHC Cre recombinase to knockout the Hif1α gene and produce offspring that either have HIF-1α in their cardiomyocytes, which will be utilized as a control, or no HIF-1α in their cardiomyocytes. The same techniques of immunofluorescence and Western blotting to fulfil aim 1 and aim 2 will then be repeated with these specific cardiomyocytes to observe if the changes to histone modifications levels between normoxic and hypoxic cardiomyocytes are dependent or independent of HIF.

Finally, to fulfill aim 3 and determine which genes specifically are being upregulated or downregulated by histone modifications during hypoxia, chromatin immunoprecipitation sequencing (ChIP-seq) will be performed and analyzed to determine the genomic landscape and distribution of H3K4me3 and H3K27me3 in normoxic and hypoxic cardiomyocytes. ChIP-seq first requires the crosslinking of protein-DNA interactions using formaldehyde. Next, the chromatin is isolated and sheared using sonication. This is then followed by immunoprecipitation of the sheared chromatin which leads to the construction of a DNA library. This library is then sequenced and bioinformatic analysis, which includes genome alignment and peak calling, is performed. This will allow for the identification of H3K27me3 and H3K4me3 locations in either a gene or between genes, i.e., intergenic regions.
Lab Trainings Completed/ Ethics Approval

The student has completed the following trainings that permit the student to research within the Shohet Laboratory: Hazardous Waste Generator Training, Laboratory Safety Training, Biosafety Training, Bloodborne Pathogens Training, Reducing Pain and Distress in Mice and Rats (CITI), Working with Mice and Rats (CITI), and Investigators Staff and Students (CITI).

Role of the Researcher

The student will be obtaining neonatal cardiomyocytes as well as breeding the HIF-conditional knockout mice under supervision at the Shohet Laboratory. The student will perform all cardiomyocyte isolation, immunofluorescence, Western blotting, and ChIP-seq experiments as well as all of the data analysis that accompany the previously mentioned experiments.

Resources and Materials

All resources and materials will be available and provided by the Shohet Laboratory at the Center for Cardiovascular Research at JABSOM.

Timetable

<table>
<thead>
<tr>
<th>Activity</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduct Research</td>
<td>Fall/Winter 2020</td>
</tr>
<tr>
<td>Outline Thesis</td>
<td>December 20, 2020</td>
</tr>
<tr>
<td>Write 1/2 of Thesis</td>
<td>January 20, 2021</td>
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<tr>
<td>First Draft Thesis</td>
<td>February 20, 2021</td>
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<tr>
<td>Final Draft Thesis and Submit to Mentor and Committee Member</td>
<td>March 20, 2021</td>
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<tr>
<td>Upload Personal Statement and Resume</td>
<td>April 1, 2021</td>
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<tr>
<td>Final Honors Project Submission</td>
<td>April 15, 2021</td>
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<tr>
<td>Undergraduate Showcase</td>
<td>April 30, 2021</td>
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<tr>
<td>Graduation</td>
<td>May 15, 2021</td>
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References


