# Climate Change Promotes Tumorigenesis Through the Epigenetic Priming of Hsp70

Matthew Binsfeld

A proposal submitted in

partial fulfillment of the requirements

for the degree of Bachelor of Arts, Biology

Luther College

Decorah, IA

December, 2025

**Abstract**

Human-induced climate change is raising global temperatures and the frequency of heat waves, potentially increasing the risk of cancer by disrupting the cellular heat shock response. Chronic exposure to elevated ambient temperatures can result in the epigenetic priming of Heat Shock Protein 70 (Hsp70), leading to sustained upregulation. Due to the anti-apoptotic functions of Hsp70, sustained expression may promote tumorigenesis and increase tumor growth rates. However, there is currently insufficient experimental evidence to establish a relationship between chronic heat exposure and increased cancer risk via epigenetic priming of Hsp70 expression. My research objective is to determine whether elevated ambient temperatures during simulated heat waves promote tumor growth in cancerous cell lines and tumorigenesis in Adenomatous polyposis coli (Apc) mice. Replicating the IPCC's predicted temperature increases, I will quantify the rates of tumor growth and tumorigenesis over 12 weeks of heat exposure. I will use RNA interference (RNAi) knockdown experiments to establish the mechanistic role of Hsp70. I hypothesize that chronic exposure to elevated temperatures increases tumor growth and tumorigenesis through the epigenetic priming of Hsp70 expression. This study will provide critical evidence of a previously uncharacterized health consequence of human-induced climate change.

**Background**

Continued rising global temperatures have the potential to increase the risk of cancer in humans by disrupting the heat shock response. It is well documented that human-induced climate change increases the frequency of extreme weather events and overall global ambient temperature (IPCC, 2023). The impact of greenhouse gases in the atmosphere is already evident in annual temperature data. The average global surface temperature over the last two decades has

been 0.99 °C warmer than in any decade from 1850 to 1900 (Lee et al., 2023). Additionally, the frequency of extreme heat and variable weather has increased over the last several decades (Perkins-Kirkpatrick and Lewis, 2020). Rising global ambient temperatures and the frequency of extreme weather events constitute critical biological variables for understanding human health and the role of the heat shock response in tumor growth and formation. Chronic exposure to elevated ambient temperatures elevates Hsp70 expression, which may promote tumor formation and growth.

Due to variations in global temperature, with the hottest and coldest locations differing by over 55°C, quantifying ambient temperature changes can be challenging ("Climate Change: Global Temperature.", 2025). The Intergovernmental Panel on Climate Change (IPCC) utilizes global temperature anomaly data to comprehend how the global average climate is evolving. By measuring temperature changes at specific locations, an average change in temperature can be calculated. In their analysis, they found an average global temperature increase of 1.1°C from the 19th century (IPCC, 2023). In response to ongoing efforts to mitigate the effects of climate change and $CO_2$ emissions, the IPCC has released a series of predictions for future temperature changes based on projected levels of greenhouse gas (GHG) emissions. These predictions range from the best-case scenario, characterized by very low GHG emissions, to the worst-case scenario, marked by very high GHG emissions. The best-case scenario would result in a 1.4°C increase in global average temperature, whereas the worst-case scenario would lead to a 4.4°C increase (IPCC, 2023). With every 0.5°C increase in global average temperatures, extreme weather events become more probable (IPCC, 2023).

A 2.7°C average global temperature increase does not imply that the whole Earth is uniformly heating up by 2.7°C. Instead, a global average temperature increase of 2.7°C

(SSP2-4.5) could mean that a city currently experiencing 10 days a year above 35°C may in the next several decades experience 30-40 days a year above 35°C (IPCC 2023). Sustained exposure to temperatures above an organism's thermoneutral point results in significant physiological burdens, as the body must divert energy to cellular defense and survival mechanisms. Human performance studies have confirmed that elevated ambient temperatures raise core temperature, a phenomenon known as systemic hyperthermia (Cramer and Jay, 2015). Human-induced increases in global ambient temperature and the frequency of aberrant weather events are increasingly implicated in disrupting key cellular responses that affect human health.

Elevated ambient heat exposure activates a multitude of cellular responses, including upregulation of Heat Shock Proteins (HSPs) (Åkerfelt et al., 2010). When a cell is exposed to heat stress, complications arise that impact its ability to perform biological processes. Proteins denature into a nonfunctional state, halting essential metabolic processes. Additionally, denatured proteins are prone to aggregating with other proteins, forming clumps of nonfunctional proteins throughout the cell. The cell has evolved to respond to heat stress and to disruptions in protein homeostasis through the heat stress response. HSPs are fundamental and highly conserved across all domains of life, including bacteria, archaea, and eukaryotes (Feder and Hofmann, 1999). Alterations in temperature affect all areas of life, and HSPs are essential for protecting cells and their mechanisms (Feder and Hofmann, 1999). HSPs are a large family of multifunctional, variably sized chaperone proteins, comprising more than 100 forms. In humans, the most commonly studied HSP is Hsp70, a 70-kDa protein responsible for refolding of disordered proteins and repression of protein aggregation (Rosenzweig et al., 2019). Hsp70 is crucial for maintaining protein homeostasis in humans (Rosenzweig et al., 2019).

When exposed to elevated ambient temperatures, Hsp70 expression is induced by Heat Shock Factor 1 (HSF1), a transcription factor that regulates gene expression (Åkerfelt et al., 2010). Transcription factors control the rate of production of a subset of proteins by binding to the promoter region of a gene. When the temperature exceeds the threshold required for homeostasis, HSF1 trimerizes and binds to DNA (Åkerfelt et al., 2010). In response to binding, HSF1 activates HSP transcription and Hsp70 production (Anckar and Sistonen, 2011). HSF1 transiently increases Hsp70 expression in response to acute heat exposure, with elevated Hsp70 expression lasting 24-48 hours (Morimoto, 1998). Acute heat exposure refers to a sudden and extreme increase in ambient temperature. As shown, global ambient temperature and the frequency of heat waves are increasing and are projected to continue rising, resulting in chronic exposure to elevated heat. Although elevated Hsp70 expression is transient, evidence is emerging that baseline HSP expression is altered by chronic heat exposure.

The conditions and variables to which the cell is exposed can alter the epigenetic state of a protein's coding region. The ability of HSF1 to bind to the promoter of Hsp70 and induce expression is dependent on the Hsp70 promoter's epigenetic state. A study examining plants suggests that cellular stress can alter chromatin state and increase the baseline expression of defense mechanisms (Pecinka and Scheid, 2012). When a cell is exposed to stress, protein expression changes in response. Under chronic stress, the cell responds by altering the chromatin state of promoter regions activated during the stress response. This alteration increases the promoter region's accessibility to transcription factor binding via histone modifications. The cell embeds its genetic conformation state, including memories of stresses and the proteins induced in expression. Modifications to histones affect the ability of transcription factors to access their target promoters. Depending on the specific type of histone modification, histones can be pulled

together, thereby limiting transcription factor-DNA interactions, or pulled apart, thereby increasing interaction.

In the case of Hsp70 and HSF1, new evidence suggests that prolonged HSP expression results from epigenetic changes (Li et al., 2024). Li et al. (2024) exposed mice to elevated temperatures (40°C) for 30 minutes daily for 10 days. Elevated HSP expression persisted beyond the 24-48 window that occurs during an acute stress response. This study suggests that when an organism is exposed to chronic increases in ambient temperature, baseline Hsp70 expression increases. This cellular response represents epigenetic priming induced by an environmental stressor (Barouki et al., 2018). However, increases in baseline stress-response mechanisms pose a challenge for maintaining cellular homeostasis. Cells out of homeostasis are at risk of mutation and uncontrolled proliferation, ultimately leading to tumor formation.

Cancerous cells are an ever-present issue in public health, and efforts to understand the characteristics of cancer cells are continuous. Research has shown that tumor cells exhibit increased Hsp70 expression (Murphy, 2013). The protective function of Hsp70 is hijacked by cancer cells to protect themselves and facilitate proliferation. Central to tumor growth is the dysfunction and downregulation of apoptosis. Throughout a cell's lifetime and during rounds of duplication, mutations accumulate and are passed on through mitosis. To protect the organism from an uncontrolled accumulation of mutations, cells are programmed to die after a variable number of divisions. Substantial evidence suggests that Hsp70 suppresses apoptotic pathways (S. Li et al., 2025). This study used RNAi to knock down Hsp70 expression to characterize the apoptotic mechanism. RNAi is a widely used method for reducing or eliminating the expression of a specific gene. When Hsp70 is upregulated, the cell’s ability to enter programmed cell death is repressed through Hsp70 cellular defense mechanisms. Hsp70 represses apoptosis by

inactivating caspases, a key agent of apoptosis. Increased Hsp70 expression has also been implicated in reducing the efficacy of anticancer therapeutics (Kunachowicz et al., 2024). Current research has not identified a relationship between Hsp70's repression of apoptosis and the human-induced increase in ambient temperature. Moreover, the impact of increased ambient temperature on tumor formation, mediated by enhanced Hsp70 expression, is poorly understood.

In this project, I aim to investigate whether exposure to simulated heat waves promotes tumor growth and tumorigenesis by increasing cellular Hsp70 expression, using both cell lines and mice as model organisms. Although $CO_2$ emissions are raising the average global temperature, the increasing frequency and intensity of heat waves are presenting possible physiological burdens on humans. Based on the above-mentioned characteristics of Hsp70, evidence suggests a potential correlation between elevated global ambient temperatures driven by $CO_2$ emissions and cancer formation. However, there is currently insufficient evidence to define a relationship or mechanism. Chronic exposure to elevated ambient temperatures may lead to tumorigenesis and accelerate tumor growth by altering the epigenetic regulation of Hsp70.

**Objectives and Specific Aims**

My overall objective is to determine whether elevated ambient temperatures during simulated heat waves promote tumorigenesis and increase tumor survival and proliferation.

1. To do this, I first aim to determine the extent to which chronic exposure to elevated ambient temperatures affects tumor growth.

I hypothesize this mechanism occurs through the epigenetic priming of Hsp70, thereby disrupting cellular apoptosis. To do this, I will measure tumor growth rates in cancerous cell lines exposed to chronic heat stress and compare them with those in cancerous cells exposed to

normal temperatures. I will use RNAi to establish the role of Hsp70 in tumor growth following heat exposure.

2. Second, I aim to determine whether chronic heat exposure increases the risk of tumorigenesis.

Similarly, I hypothesize that this mechanism occurs through the epigenetic priming of Hsp70, leading to the formation of a pro-tumorigenic environment. I will measure tumor formation rates in mice exposed to chronic heat stress and compare them with those of control mice exposed to normal temperatures. I aim to use RNAi to characterize the mechanistic role of Hsp70 in tumorigenesis in response to chronic elevated heat exposure. By replicating the IPCC's projected changes in ambient temperatures and heatwave frequencies, this study aims to provide policymakers with evidence on the potential health impacts associated with climate change.

## Approach & Methods

### Rationale

To investigate the relationship between chronic heat exposure and tumor success, I will use cell lines to assess tumor growth and mice to assess tumor formation. To ensure this study's applicability, the IPCC's predicted global temperature increases will be used to establish experimental groups for both the cell line and the mouse protocols. Additionally, to replicate the predicted heat wave frequency and length, the cell line and mice experiments will each be carried out for 12 weeks. To extend beyond transient acute heat exposure into epigenetic state shifts, a 12-week period will be sufficient to ensure the response is not purely acute. A sustained, non-lethal level of heat exposure over an extended period enables examination of long-term adaptations in Hsp70 expression.

**Cell Line Protocol**

To determine the effect of chronic exposure to elevated ambient temperatures on tumor growth, I will expose cancerous fibroblast cell lines to elevated ambient temperatures, replicating the projected temperature changes from the IPCC (2023). A control group incubated at 37°C, the standard core body temperature, will provide a baseline for quantifying tumor growth rate. I will prepare three experimental groups to replicate the IPCC's predictions of future GHG emissions under SSP1-1.9, SSP2-4.5, and SSP5-8.5. I will culture the experimental groups at 38.4°C, 39.7°C, and 41.4°C, respectively, to align with the best-case (SSP1-1.9), intermediate (SSP1-4.5), and worst-case emission (SSP1-8.5) scenarios. To simulate chronic exposure to elevated temperature, cells will be incubated for 12 weeks. Each week, I will quantify proliferation and cell health using MTT/MTS assays and cell counting. MTT/MTS assays are commonly used to measure cell viability, proliferation, and cytotoxicity. Cell death will be quantified using Trypan Blue exclusion and flow cytometry. In addition to measuring tumor growth, I will also quantify Hsp70 expression during the experiment. Each week, RT-qPCR will be used to quantify Hsp70 expression. Over 12 weeks of cell culture, I will collect data on cell proliferation, total cell count, cell death, and Hsp70 expression. The data will be examined for apparent, temperature-dependent effects on all measured variables.

**Cell Line RNAi Assay**

To investigate the mechanism of tumor growth in response to chronic heat exposure, I will use RNAi to generate cell lines lacking Hsp70 expression. By directing RNAi molecules to degrade mRNA encoding Hsp70, resulting in little to no Hsp70 expression, the role of Hsp70 can be identified. Following the same temperature conditions and controls as in the cell line protocol, the RNAi assay will be used to identify the mechanism underlying temperature-induced tumor

growth. This assay is intended to determine whether Hsp70 plays a mechanistic role in tumor growth rate.

**Mice Protocol**

To determine the effect of upregulated Hsp70 on tumorigenesis, adenomatous polyposis coli (Apc) mice will be exposed to a chronically elevated ambient temperature and analyzed for tumor formation. These Apc mice are genetically engineered to be highly susceptible to intestinal tumorigenesis and are commonly used in tumorigenesis assays. To establish a control, mice will be housed in a 30°C chamber, representing their thermoneutral baseline. Experimental groups will include mice housed in chambers at 31.4°C, 32.7°C, and 34.4 °C. Over 12 weeks, Hsp70 expression, body weight, food intake, and water consumption will be quantified weekly. Hsp70 expression will be quantified with RT-qPCR. After 12 weeks, mice will be humanely euthanized for a comprehensive analysis. The intestine will be dissected, and the number, mass, and location of all visible tumors will be recorded.

**Mice RNAi Assay**

To investigate Hsp70 expression as a mechanism of tumorigenesis, RNAi will be used to knock down Hsp70 in the intestinal epithelial cells of adenomatous polyposis coli mice. Using the same experimental temperatures and controls as in the mice protocol above, tumor formation in Hsp70 knockdown mice and canonical mice will be compared to determine whether primed Hsp70 expression affects tumorigenesis.

**Alternate Approach**

If the previously mentioned aims do not yield significant results, I will pursue an alternative aim. To determine how chronic heat stress affects a tumor's response to a therapeutic, I will measure the fitness of a tumor cell line exposed to chronic heat stress and the therapeutic,

compared with a control tumor cell line exposed to the same therapeutic. If the alternative aim yields results, I will proceed to use mice as the model organism and replicate the Mice Protocol.

**Limitations**

This study aims to investigate how chronic heat stress affects the health and survival of both cell lines and mice, with the intention that the findings could be broadly extended to questions in human health. Although the protein-coding regions of mice and humans are 85% identical, extending findings from one organism to another requires careful consideration of the study's context (“Why Mouse Matters.”, 2025). The transition between mouse and human biology involves considering key factors. First, mice have higher metabolic rates and distinct thermoregulatory pathways compared to humans. Differences in metabolism may result in complex and multivariable responses to heat stress, and their effects cannot always be quantified or predicted. This model isolates the biological effect, but may not fully capture the human reality. Second, using adenomatous polyposis coli mice represents only one pathway to cancer, limiting the applicability of findings to other forms of cancer. The difficulty of measuring tumor formation over a short period limits the study's applicability to human health.

In determining whether heat stress leads to tumorigenesis, a cancerous cell line cannot be used. To assess the effect of Hsp70 on tumor formation, a multicellular organism capable of developing tumor cells is required. Given the ethical considerations involved in studying tumor formation, I intend to minimize the number of mice used while maintaining a robust experimental design.

## Significance

### Intellectual Merit

A new understanding of Hsp70's roles in tumor growth and formation will enable better cancer prevention as the climate continues to warm. My current project focuses on the relationship between human-induced climate change and the effects Hsp70 has on tumors, with the potential to reveal previously unknown connections. For lawmakers, this research could help illustrate the real-life impacts of climate change. For clinicians and doctors, understanding the implications of heat stress on human health may lead to improved prevention and assessment of cancer. With the ultimate goal of advancing knowledge of cancer mechanisms, this study aims to efficiently characterize the role of Hsp70 in tumor growth and formation, thereby extending findings from cell lines and mouse models to human health.

**Broader Impacts**

This project will provide a powerful avenue for education and training in cell culture and in performing experiments with mice. I will conduct the cell culture experiments with the assistance of three undergraduate students seeking experience in biochemical research and experimental design. The various methods of quantification in both the cell line protocol and the mice protocol will provide students opportunities for growth in biological analysis. Additionally, I plan to disseminate my results to both the academic community at research symposia and to lawmakers. As the climate continues to change, this study has the potential to raise awareness of the causes of cancer and reduce the overall incidence of cancer in humans. Expanding understanding of the specific health implications of climate change may induce action at a level not yet observed.

**Literature Cited**

Åkerfelt, Malin, et al. “Heat Shock Factors: Integrators of Cell Stress, Development and Lifespan.” *Nature Reviews Molecular Cell Biology*, vol. 11, no. 8, July 2010, pp. 545–55. https://doi.org/10.1038/nrm2938.

Allan, Richard P., et al. “Summary for Policymakers.” Climate Change 2021: The Physical Science Basis, edited by V. Masson-Delmotte et al., Cambridge UP, 2021, pp. 3–32. https://doi.org/10.1017/9781009157896.001.

Anckar, Julius, and Lea Sistonen. “Regulation of HSF1 Function in the Heat Stress Response: Implications in Aging and Disease.” *Annual Review of Biochemistry*, vol. 80, no. 1, Mar. 2011, pp. 1089–115. https://doi.org/10.1146/annurev-biochem-060809-095203.

Barouki, R., et al. “Epigenetics as a Mechanism Linking Developmental Exposures to Long-term Toxicity.” *Environment International*, vol. 114, Feb. 2018, pp. 77–86. https://doi.org/10.1016/j.envint.2018.02.014.

“Climate Change: Global Temperature.” NOAA Climate.gov, 29 May 2025, www.climate.gov/news-features/understanding-climate/climate-change-global-temperature.

Cramer, Matthew N., and Ollie Jay. “Explained Variance in the Thermoregulatory Responses to Exercise: The Independent Roles of Biophysical and Fitness/Fatness-related Factors.” *Journal of Applied Physiology*, vol. 119, no. 9, Aug. 2015, pp. 982–89. https://doi.org/10.1152/japplphysiol.00281.2015.

Du, Sihan, et al. “Advances in the Study of HSP70 Inhibitors to Enhance the Sensitivity of Tumor Cells to Radiotherapy.” *Frontiers in Cell and Developmental Biology*, vol. 10, Aug. 2022, https://doi.org/10.3389/fcell.2022.942828.

Feder, Martin E., and Gretchen E. Hofmann. “HEAT-SHOCK PROTEINS, MOLECULAR CHAPERONES, AND THE STRESS RESPONSE: Evolutionary and Ecological Physiology.” *Annual Review of Physiology*, vol. 61, no. 1, Mar. 1999, pp. 243–82. https://doi.org/10.1146/annurev.physiol.61.1.243.

IPCC. Climate Change 2023: Synthesis Report. Edited by Core Writing Team et al., 2023, pp. 35–115. https://doi.org/10.59327/IPCC/AR6-9789291691647.

Kunachowicz, Dominika, et al. “Heat Shock Proteins, a Double-Edged Sword: Significance in Cancer Progression, Chemotherapy Resistance and Novel Therapeutic Perspectives.” *Cancers*, vol. 16, no. 8, Apr. 2024, p. 1500. https://doi.org/10.3390/cancers16081500.

Lacis, Andrew A., et al. “Atmospheric CO 2 : Principal Control Knob Governing Earth’s Temperature.” *Science*, vol. 330, no. 6002, Oct. 2010, pp. 356–59. https://doi.org/10.1126/science.1190653.

Li, Can, et al. “Heat Acclimation Mediates Cellular Protection via HSP70 Stabilization of HIF-1α Protein in Extreme Environments.” *International Journal of Biological Sciences*, vol. 21, no. 1, Dec. 2024, pp. 175–88. https://doi.org/10.7150/ijbs.103122.

Li, Sicheng, et al. “Role of Heat Shock Proteins in Response to Temperature Stress and Their Effect on Apoptosis in Drosophila Melanogaster.” *International Journal of Biological Macromolecules*, Feb. 2025, p. 141320. https://doi.org/10.1016/j.ijbiomac.2025.141320.

Lee, Hoesung, et al. “Climate Change 2023: Synthesis Report.” Climate Change 2023: Synthesis Report, edited by Core Writing Team et al., illustrated by Korea Meteorological Administration, report, World Meteorological Organization, 2023, pp. 1–34. https://doi.org/10.59327/IPCC/AR6-9789291691647.001.

Morimoto, Richard I. “Regulation of the Heat Shock Transcriptional Response: Cross Talk Between a Family of Heat Shock Factors, Molecular Chaperones, and Negative Regulators.” *Genes & Development*, vol. 12, no. 24, Dec. 1998, pp. 3788–96. https://doi.org/10.1101/gad.12.24.3788.

Murphy, M. E. “The HSP70 Family and Cancer.” *Carcinogenesis*, vol. 34, no. 6, Apr. 2013, pp. 1181–88. https://doi.org/10.1093/carcin/bgt111.

Pecinka, A., and O. Mittelsten Scheid. “Stress-Induced Chromatin Changes: A Critical View on Their Heritability.” *Plant and Cell Physiology*, vol. 53, no. 5, Mar. 2012, pp. 801–08. https://doi.org/10.1093/pcp/pcs044.

Perkins-Kirkpatrick, S. E., and S. C. Lewis. “Increasing Trends in Regional Heatwaves.” *Nature Communications*, vol. 11, no. 1, July 2020, https://doi.org/10.1038/s41467-020-16970-7.

Rosenzweig, Rina, et al. “The Hsp70 Chaperone Network.” *Nature Reviews Molecular Cell Biology*, vol. 20, no. 11, June 2019, pp. 665–80. https://doi.org/10.1038/s41580-019-0133-3.

“Why Mouse Matters.” Genome.gov, www.genome.gov/10001345/importance-of-mouse-genome#:~:text=On%20average%2C%20the%20protein%2Dcoding,because%20of%20their%20greater%20similarity.