DNA Methylation in Autophagy-associated Genes and Risk of Prostate Cancer

Jimmy Ren, Dr. Yinan Zheng, Northwestern University

Autophagy

- Autophagy is a catabolic process that degrades and recycles damaged organelles and macromolecules within the cell
  - Autophagy is generally seen survival mechanism.
- Autophagy plays a key role in preventing diseases
  - It have also shown autophagy to be a tumor suppressive mechanism

DNA Methylation

- DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine
  - DNA methylation regulates gene expression
- These epigenetic modifications, such as DNA methylation have shown to be a part of carcinogenesis
- Promoter methylation may silence crucial genes that have been shown to regulate autophagy in cancer.

Prostate Cancer

- Prostate cancer is the most frequent tumor found in men worldwide
- Previous studies have shown clear indications that stable epigenomic changes occur in cells of prostate cancer.
- One of the most occurring events in prostate cancer is DNA methylation.
- There may be a strong relationship in biological mechanisms and pathways between promoter methylation in autophagy genes and prostate cancer.

NAS Data

- The Normative Aging Study (NAS) was established by the US Department of Veteran Affairs (VA) in 1963
- 981 participants died and 470 were lost to follow up.
- Participants were recalled for clinical examinations every 3–5 years
- Starting in 1999, these included 7-ml blood samples for DNA analysis.
- This study was approved by the Institutional Review Boards of all participating institutions, and all participants provided written consent.

NAS Data (cont.)

- Only cancer-free participants and participants with prostate cancer were considered
- 36 (28.8%) had prostate cancer, while the remaining population had digestive, skin, respiratory, and other cancers.
- The remaining population of 402 participants was further limited due to the nature of the all-male cohort.
- The participants were then split into 3 groups based off the amount of years before their diagnosis date: 0-4 years, 4-8 years, and 8+ years from their baseline date.
- Information on cancer diagnosis was obtained from questionnaires and confirmed via review of medical records.

Initial Analysis

- Each group included the cancer-free participants for a comparison analysis between the beta values that measure DNA methylation.
- 354 cancer-free participants, 0-4 years had 28 cancer participants, 4-8 years had 9 cancer participants, and 8+ years had 6 cancer participants.
- Each group was run through a robust linear regression and linear regression model to get better insight into the significant CpG sites.
- Only the CpGs that were significant between at least 2 of the groups were considered and compared to understand the DNA methylation changes.

Initial Analysis (cont.)

- All models were adjusted for age, education, BMI, and cell proportion.
- Each group included the cancer-free participants for a comparison analysis between the beta values that measure DNA methylation.
- Each group was run through a robust linear regression and linear regression model to get better insight into the significant CpG sites.
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Final Analysis

- The robust linear regression model was later used to compare between the groups.
- Smoke years and alcohol consumption did not appreciably affect our results, prompting their exclusion.
- Participants missing any data for outcome was discluded
- Figures were generated using R v3.5.1.
- Statistical significance threshold was set to $p = 0.01$.
- Sensitivity analyses and KEGG Pathway tests were run in order to gain better insight into the significance of the results.
- All models were adjusted for age, education, BMI, and cell proportion.

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Sensitivity and Genomic Analysis

- The purpose of the sensitivity analysis is to test the robustness of the model.
- A crude model was compared with the adjusted model to verify the significance of the results.
- Each showed a strong, positive correlation with $r > 0.9$.
- Each CpG site showed a consistency between their respective groups.
- For Years 8+ and Years 0-4, the sensitivity analysis showed a stronger correlation ($r=0.959$, $r=0.969$) compared to Years 4-8 ($r = 0.942$).
- The genomic analysis failed to report anything significant to report to further strengthen the results.

KEGG Pathway Analysis

- Using the significant 22 CpG sites, 60 gene pathways were identified by using the KEGG and statistical tests.
- 15 of the pathways were significant (p-value < 0.05).
- The most significant pathways include necroptosis, calcium signaling pathway, insulin secretion, circadian entrainment, gastric acid secretion, and aldosterone synthesis and secretion ($p < 0.01$)
- Mechanisms and regulation of necroptosis affects tumorigenesis
- Calcium signaling has been showing signs of providing an effective diagnosis and treatment of prostate cancer.

Conclusions

- The robust linear model located 2,113 significant CpG sites.
- The 22 significant and directionally consistent CpG sites were considered further, and were limited down to 12 genes to consider only the CpG sites that lied on the promoter region.
- Previous studies have shown significant results for the 12 genes that the CpG sites lie on.
    - HSP27, SQSTM1, TFEB, REL, VDAC1 remain consistent with my findings
- KRT74z, PRKCZ, SNORA84, TMEM49, SLC7A6, and BRSK2 are novel biomarkers that need further research in order to verify their autophagic effects in prostate cancer.

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Conclusions (cont.)

- The work in the field of autophagy and its relationship to cancer is growing, as the complex associations and relationships between autophagy and cancer are being explored.
- This paper seeks to further clarify the relationship by verifying some previously known mechanisms
- This prospective study design allows pediatricians and pharmaceutical companies to utilize this information and target these biomarkers beforehand to develop a better prognosis and treatment plan.

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Future Research

- Research into both calcium signaling and necroptosis molecular mechanisms could potentially yield new biomarkers for researchers to utilize.
- The relationships between the functionality of autophagy and these pathways to prostate cancer to create effective prognosis and treatment methods for the future.

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