Diet, Cancer, and Epigenetics

• Author’s Name: Abbas, A., Hall, J. A., Patterson, W., Al-Mulla, F., Wilson, J., Applegate, M., Ho, E., and Georgel, P.T.

• Abstract: Prostate cancer is currently the second most common cause of death from cancer in men in the United States. Epidemiologic studies have linked diets rich in sulforaphane (an isothiocyanate isolated from cruciferous vegetables) with a marked reduction in prostate cancer (PCA) risk. Sulforaphane (SFN), delivered to PCA cell lines at doses compatible with a daily diet, leads to an inhibition of cell growth and an increased transcription/translation of tumor suppressors, such as the cell cycle regulator p21\textsuperscript{CIP1}. Using these cell lines, we have demonstrated that the expression of human Telomerase Reverse Transcriptase (hTERT), a known cancer marker, was down-regulated by SFN treatment. An epigenetic effect associated with SFN histone deacetylase (HDAC) inhibitor activity has been demonstrated in PCA cell lines. To further investigate this epigenetic connection, we treated the PCA cell lines LNCaP and DU-145 with 15µM SFN for 24 hours, and recorded that, in addition to the expected increase in global histone H3 K18 acetylation, histone H3K4di-methylation levels (another predictor of high risk of PCA recurrence) were also increased. Initial chromatin-immunoprecipitation assays using antibodies against these two modified histones appear to indicate a change in distribution over the promoter regions of hTERT, suggesting a coordinated event. As a complement to our initial analysis of the epigenetic changes induced by SFN treatment, we have initiated a microRNA (miR) expression study. The initial results indicate that a subset of miRs was differentially expressed. Upon closer analysis, several of these miRs are known to be important for PCA development, as well as being linked to the regulation of expression of proteins involved in chromatin modifications and remodeling. These changes in pattern of histone modifications, and miR expression mediated by SFN, combined with the remodeling that we observed in chromatin structure strongly suggest a complex mode of epigenetic regulation for the oncogene hTERT.

• Keywords: Diet, Prostate Cancer, Epigenetics

• Full text, with all subsections and illustrations
Dietary regimen can play a role in reducing cancer incidence, as indicated by epidemiologic evidence (American Institute for Cancer Research’s publication). In addition, specific compounds present in various diets have been shown to have a strong potential as therapeutic agents. The list of such compounds and their effects on various types of cancers is increasing every year. As these compounds are unlikely to be responsible for changes in the genetic material (mutations), the mode of action is likely to be related to epigenetic mechanisms. A family of compounds involved in such mechanism includes dietary polyphenols (Abbas et al., 2013). The various polyphenols listed in that publication have been associated with changes in epigenetic markers such as DNA methylation status, histone post-translational modifications (PTMs), chromatin remodeling, and microRNA expression profile. Studies involving in vitro and in vivo models confirm this epigenetic connection, demonstrating that dietary compounds were able to affect the epigenome in a direct manner (sulforaphane, and genistein acting as histone deacetylase or HDAC inhibitors), as other compounds, such as omega-3 fatty acids may act in a more complex and indirect manner. A variety of histone PTMs have been associated with specific cellular functions, from
transcription activation/repression transition to imprinting. In addition to the changes in chromatin composition, epigenetic regulations can mediate gene expression levels by affecting the extent of chromatin compaction (Georgel P.T., 2007), showing a direct correlation between the level of transcription repression and chromatin folding. Our current studies have been focused on the effect of the dietary polyphenol sulforaphane (SFN) and prostate cancer (PCa). The connection between SFN, a HDAC inhibitor and PCa recurrence was hypothesized to be mediated by epigenetic changes (histone PTMs), based on the research by Seligson and co-workers (2005). This research group demonstrated that the presence of specific histone PTMs, including acetylation of histone H3 Lysine 18 (H3K18ac) and di-methylation of histone H3 Lysine 4 (H3K4me2) could be used as a predictor for PCa recurrence.

SFN was first characterized as a potential drug against various types of cancer through its ability to induce apoptosis and affect the cell cycle regulation in PCa cell lines, but, initially, the contribution of its HDAC inhibitor activity was not fully understood.

This epigenetic connection was suggested as studies focused on specific genes associated with PCa, such as the tumor suppressor p21 and NF-kB, which appeared to be more specifically targeted (Ho et al., 2009). Our own research revealed evidence also suggesting a SFN-dependent role for two proteins involved in chromatin compaction (the methyl DNA-binding protein and chromatin compactor MeCP2, and Brg1, the ATPase sub-unit of the Swi/SNF chromatin-remodeling complex). This combination of evidence lead us to hypothesize that SFN affects gene expression in PCa cells through epigenetic changes (DNA methylation, chromatin structure and composition, histone modifications, chromatin-associated proteins), and additional work also suggested a connection with changes in cell’s microRNA expression pattern.

PCa cells often re-initiate expression of the oncogene human telomerase reverse-transcriptase (hTERT) leading to telomere elongation (a property associated with cell’s immortality issue). Our experiments using SFN treatment of hTERT-positive PCa cells demonstrated that exposure of PCa cells to this polyphenol resulted in a decrease in hTERT expression and activity. To confirm our hypothesis and demonstrate the connection between SFN treatment and epigenetic modifications, we initially focused on histone PTMs associated with PCa (H3K18ac and H3K4me2, see map in Lund and van Lohuizen, 2004).

Our study showed that, as expected and due to its HDAC activity, SFN treatment of various hTERT-positive PCa cell lines resulted in increased histone H3 and H4 acetylation (both globally and on specific Lysine residues), but no change in control prostate cells. In addition, SFN treatment resulted in a coordinated increase in histone H3 K4 di-methylation. We confirmed that the SFN-induced epigenetic effect was not just a global occurrence, but that it was observed specifically over the promoter and early coding region of the hTERT gene. We also confirmed that SFN could affect the level of chromatin conformation by monitoring the average distance between adjacent nucleosomes (Nucleosome Repeat length or NRL) over the hTERT promoter and early-coding region.

In addition, we confirmed that the protein MeCP2, known to promote chromatin compaction (Georgel et al., 2003) was recruited specifically at regions where the NRL was affected by SFN
treatment. The MeCP2 recruitment was correlated with changes in H3K4me2 distribution over the hTERT promoter (as expected based on previous results from Thambirajah et al., 2012). From these results, we conclude that SFN treatment appears to affect specific epigenetic modifications, based on our investigation of the pattern of histone acetylation showing specificity (+/- SFN). The SFN-mediated effect appears to coordinate the pattern of histone methylation with that of histone H3K18 acetylation. The changes in distribution of these two histone PTMs can, in turn affect chromatin compaction by promoting the recruitment of MeCP2 at specific location over the hTERT promoter region.

This SFN’s mechanism of action appears to be complex and may show a certain amount of cell specificity, as different PCa cell types may use the same types of epigenetic modifications in a slightly different manner to regulate gene expression depending on cancer stage (androgen positive or negative). Ultimately, we expect that the epigenetic information related to the mode of action of dietary compounds, such as SFN will provide new avenues to specifically target PCa cells and lead to new therapies.

**References**


The epigenetic potential of dietary polyphenols in prostate cancer. Abbas, A., Patterson, W., and Georgel P.T. Biochemistry and Cell Biology 2013 Vol. 91.361-368


Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. Ho E, Clarke JD, Dashwood RH. J Nutr. 2009 Dec;139(12):2393-6


**Author’s biography:** Dr. Georgel received his PhD training investigating various aspects of gene expression regulation involving chromatin structure and function at Oregon State University, under the supervision of Dr. Ken van Holde. His research made use of biophysical techniques to investigate chromatin folding. His postdoctoral training at the National Institute of Health (Bethesda, MD, USA) provided him with further expertise on molecular aspects of chromatin remodeling and regulation of gene expression. More recently, he has been involved in the development of a unique biophysical method used to assess chromatin folding using in vivo-
assembled nucleosome arrays. This technique can be used as part of his current research aimed at identifying the role of sulforaphane (a poly-phenolic compound found in cruciferous plants) in epigenetic events associated with chromatin folding in prostate cancer. He has also collaborated with several US researchers on other aspects of cancer and diet-mediated epigenetic events. His work performed with Drs. Niles and Sollars (Marshall University, WV, USA) has resulted in a publication on the role of retinoic acid in melanoma. In addition, Dr. Hardman (Marshall University, WV, USA) and Dr. Georgel have started to work on investigating trans-generational epigenetic effects mediated by omega-3 fatty acid-rich diet on breast cancer.

• Author’s postal and email address:
Philippe Georgel, PhD
Marshall University
Department of Biological Sciences
Cell Differentiation and Development Center
1 John Marshall Drive
Huntington WV 25755 USA
Georgel@marshall.edu