An Insight Into Curcumin Derivatives in Reactivation of Nrf2 Anti-oxidative Stress Signaling in Mouse Prostate Cancer TRAMP C1 Cells

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Purpose
In the United States, prostate cancer is the most common non-skin male cancer sub-type. Cancer development has been linked to epigenetic modifications of cancer oncogenes and tumor suppressor genes. We have demonstrated that the progression of prostate tumors in TRAMP mice is highly associated with hypermethylation in Nrf2 promoter region and accompanied reduced transcription of Nrf2 and Nrf2 downstream genes. We have previously demonstrated curcumin is effective against the growth and progression of prostate tumor in TRAMP mice and this effect is at least in part, through epigenetic modification of the Nrf2 gene with its subsequent induction of the Nrf2-mediated anti-oxidative stress cellular defense pathway. E10 and F10 are both synthetic curcumin analogs and demonstrates powerful anti-prostate cancer activity. However, the mechanism is not clear, we aimed to investigate and compare the potential effects of the two curcumin related compounds on reactivating Nrf2 centered anti-oxidative stress pathway in TRAMP C1 cells.

Methods
The stably transfected HepG2-C8 cells expressing the ARE-luciferase vector were treated with FN1, curcumin and sulforaphane (SFN) to compare their effects on Nrf2-ARE pathways. TRAMPC1 cells were treated with E10, F10, 5-azadeoxycytidine (5-aza, a DNMT inhibitor) and trichostatin A (TSA, an HDAC inhibitor) for 3 days. Real-time quantitative PCR and Western-blotting were utilized to study the influence on endogenous Nrf2 and its downstream genes. Bisulfite genomic sequencing and methylation DNA immunoprecipitation (MeDIP) were used to study the methylation profile of Nrf2 promoter. Anchorage-independent colonies formation assay was performed to test the tumorigenicity. Epigenetic modifying enzymes, including DNMTs and HDACs, were investigated by western-blotting.

Results
Comparing with curcumin and SFN, F10 is more potent in activating Nrf2-ARE pathways in transfected HepG2-C8 cells. In TRAMP-C1 cells, both E10 and F10 enhanced the mRNA and protein expression of endogenous Nrf2 and Nrf2 downstream genes, such as HO-1, NQO1, and UGT1A1. Both E10 and F10 could reduce the expression of Keap-1. Both E10 and F10 at the concentrations of 50 and 100nM could significantly suppressed colony formation of TRAMP C1 cells. Bisulfite genomic sequencing revealed that 100nM F10 treatment for 3 days, similarly like 5aza and TSA, could decrease the level of methylation of the first five CpGs of the Nrf2 promoter. MeDIP assay double confirmed finding. F10 also downregulates epigenetic modifying enzymes, especially DNMT1, DNMT3b and HDAC1, HDAC4, HDAC7 and HDAC8. E10 has not shown demethylation effect but inhibition the protein expression of Keap-1, HDAC4 and HDAC7.

Conclusion
In conclusion, our results were the first to suggest that curcumin derivatives E10 and F10 could activate Nrf2-ARE pathway, enhance expression of phase II detoxifying and antioxidative stress enzymes, including HO-1, NQO1, and UGT1A1 and inhibit colony formation of TRAMP C1 cells. The reactivation effects of them were at least partially if not purely from epigenetically modification of demethylation of Nrf2 promoter and/or inhibiting of HDACs protein expression. E10 and F10 are proven to be novel potential cancer chemopreventive agents for prostate cancer.