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Epigenetic regulation of oncogenic signaling pathways in breast cancer cells upon exposure to dietary polyphenols

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Introduction: Alterations in DNA methylation in cancer underlie up-regulation of genes with oncogenic functions[1]. The NOTCH pathway is often overactive in breast cancer and plays roles in cancer development and progression[2]. However, the mechanisms of NOTCH regulation in mammary carcinogenesis remain unclear which hinders the development of effective therapeutic approaches. Interestingly, certain dietary compounds such as polyphenols suppress the NOTCH pathway in cancer and were shown in our study to modify epigenetic marks in genes positively regulating the pathway, including MAML2.

Method: In the present study, using two polyphenols, resveratrol from grapes and pterostilbene from blueberries, we investigate the role of DNA methylation in regulation of NOTCH and other oncogenic pathways in cancer. Non-invasive MCF10CA1h and invasive MCF10CA1a human breast cancer cell lines were used as an experimental model. Following genome-wide DNA methylation analysis with Illumina-450K array, pyrosequencing and QPCR were performed to assess methylation and expression of genes. Chromatin immunoprecipitation was applied to determine binding of epigenetic enzymes to DNA.

Results: We found 4,293 CpG loci differentially methylated upon 9-day treatment of MCF10CA1h breast cancer cells with 15μM resveratrol as compared to untreated cells (differential methylation>0.05, $p<0.05$). 60% of those CpG sites were hypermethylated and located within genes functionally associated with oncogenic signaling pathways, including NOTCH. Resveratrol led to increased methylation of enhancer region of MAML2 that is a coactivator of NOTCH target genes. Increase in MAML2 methylation was confirmed by pyrosequencing in both non-invasive and invasive breast cancer cells. Pterostilbene exerted similar effects. Along with methylation of MAML2 enhancer, the compounds decreased MAML2 expression and downregulated NOTCH target genes. Depletion of MAML2 mimicked polyphenols effects leading to suppression of NOTCH pathway. The most profound effects in response to polyphenols were observed in invasive cells where further analyses revealed increase in histone repressive marks and decrease in activating marks at MAML2 enhancer. The condensed chromatin structure was associated with binding of DNMT3B to MAML2, which suggests the role of DNMT3B in increased methylation of MAML2 upon polyphenol treatment. Similar epigenetic regulation was confirmed for WNT and Hedgehog oncogenic pathways.

Conclusion: Our results provide insight into regulation of oncogenic signals in cancer and support for epigenetic-targeting strategies as an effective anti-cancer approach. This study was supported by PCCR, Indiana CTSI (UL1TR001108), WGHI, USDA (Hatch: 1005656) granted to BS.

References: 1. Stefanska B *et al.* (2011). *Cancer Res* **71**: 5891-5903. 2. Suman S *et al.* (2013). *Br J Cancer* **109**, 2587-2596.