

## Women's Health Research Day

" Why SPARC Methylation matters: Focus on cancer and regenerative Medicine"

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Tilte: Epigenetic Regulation of SPARC In Ovarian Cancer

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Ovarian cancer is the second most common gynecologic cancer and one of the leading causes of cancer deaths in women. Survival to incidence ratio is still poor and overall cure rate remains very low. Our lab has identified Secreted Protein Acidic and Rich in Cysteine (SPARC) as a tumor suppressor in ovarian cancer. SPARC expression is downregulated in the cancerous compartment been with advanced disease stage. Importantly, SPARC has been demonstrated to be epigenetically silenced in ovarian cancer. SPARC promoter is methylated in 68% of high grade serous ovarian cancer, and in established ovarian cancer cell lines. Mechanistic studies indicated that inflammation mediated by p65RelA subunit of nuclear factor- $\kappa$ B (NF $\kappa$ B) induce SPARC promoter methylation. Therefore, we hypothesized that a persistent inflammation in the tumor microenvironment activates NF $\kappa$ B (specifically p65RelA subunit) that recruits DNMT (DNA (cytosine-5)-methyltransferase) to SPARC promoter leading to its silencing. To test the hypothesis, we performed immunostaining of p65Rel-A, DNMTs (1, 3a and 3b) in ovarian cancer tissue microarrays (TMA). We also determined SPARC protein and transcript expression in consecutive sections of the ovarian TMA by immunohistochemistry and fluorescence *in situ* hybridization, respectively. Rel-A colocalization with DNMTs were studied using proximity ligation assays. We used digital image analysis software (visiopharm) to analyze TMA with immunofluorescence stain. We determined the percentage and intensity of positive fluorescence signals in tumor cells *versus* surrounding stroma or the tumor region of interest (ROI). Our results indicated that SPARC expression negatively correlated with tumor stage and nuclear expression of p65RelA, DNMT1, 3a and 3b, respectively. Analysis of co-localization of Rel-A with each of DNMTs provided a mechanistic insight on the regulation of SPARC in tissues of ovarian cancer patients. Our data further highlight the potential of utilizing SPARC as a novel biomarker or therapeutic target in ovarian cancer.

*Supported by Marsha Rivikin Ovarian Cancer Foundation and WFBMC-CTSI-ignition funds (NS).*