Published in final edited form as:
Gynecol Oncol. 2014 February ; 132(2): 462–467. doi:10.1016/j.ygyno.2013.12.024.

LINE1 and Alu repetitive element DNA methylation in tumors and white blood cells from epithelial ovarian cancer patients

Stacey N. Akers[a], **Kirsten Moysich**[b], **Wa Zhang**[c,d], **Golda Collamat Lai**[c], **Austin Miller**[e], **Shashikant Lele**[a], **Kunle Odunsi**[a,f,g], and **Adam R. Karpf**[c,d,*]

[a]Department of Gynecologic Oncology, Roswell Park Cancer Institute, Buffalo, NY

[b]Department of Cancer Prevention and Pathology, Roswell Park Cancer Institute, Buffalo, NY

[c]Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY

[d]Eppley Institute, Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE

[e]Department of Biostatistics, Roswell Park Cancer Institute, Buffalo, NY

[f]Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY

[g]Center for Immunotherapy, Roswell Park Cancer Institute, Buffalo, NY

Abstract

Objective—We determined whether DNA methylation of repetitive elements (RE) is altered in epithelial ovarian cancer (EOC) patient tumors and white blood cells (WBC), compared to normal tissue controls.

Methods—Two different quantitative measures of RE methylation (*LINE1* and *Alu* bisulfite pyrosequencing) were used in normal and tumor tissues from EOC cases and controls. Tissues analyzed included: i) EOC, ii) normal ovarian surface epithelia (OSE), iii) normal fallopian tube surface epithelia (FTE), iv) WBC from EOC patients, obtained before and after treatment, and v) WBC from demographically-matched controls.

Results—REs were significantly hypomethylated in EOC compared to OSE and FTE, and *LINE1* and *Alu* methylation showed a significant direct association in these tissues. In contrast, WBC RE methylation was significantly higher in EOC cases compared to controls. RE methylation in patient-matched EOC tumors and pre-treatment WBC did not correlate.

Conclusions—EOC shows robust RE hypomethylation compared to normal tissues from which the disease arises. In contrast, RE are generally hypermethylated in EOC patient WBC compared to controls. EOC tumor and WBC methylation did not correlate in matched patients, suggesting that RE methylation is independently controlled in tumor and normal tissues. Despite the significant differences observed over the population, the range of RE methylation in patient and control WBC overlapped, limiting their specific utility as an EOC biomarker. However, our data

*Corresponding author at: Eppley Institute, Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, 985950 Nebraska Medical Center, Omaha, NE, USA, 68198-59590. Phone: 402-559-6115, Fax: 402-559-4651, adam.karpf@unmc.edu.

Conflicts of Interest: The authors declare no conflicts of interest.

demonstrate that DNA methylation is deranged in normal tissues from EOC patients, supporting further investigation of WBC DNA methylation biomarkers suitable for EOC risk assessment.

Introduction

Epithelial ovarian cancer (EOC) is the most common form of ovarian cancer and most lethal gynecologic malignancy; in the United States approximately 22,000 new EOC cases and 14,000 deaths are expected in 2013 [1]. Most EOC patients are diagnosed with advanced disease, which is in large part a consequence of the lack of useful diagnostic biomarkers. Patients with advanced stage disease have a five year survival of 15-20%, demonstrating the need for early detection to improve treatment responses and overall survival [2].

Cytosine DNA methylation (DNA methylation) is a covalent modification targeted to CpG dinucleotides in mammals. DNA methylation is essential for mammalian development, genomic imprinting, and X chromosome inactivation, and DNA methylation patterns are faithfully copied through mitosis, making it an epigenetic mark [3]. In cancer, DNA methylation alterations are common and include both gains (hypermethylation) and losses (hypomethylation), often concurrently [4]. Cancer-specific DNA hypomethylation is often “global” in nature, and is manifested by reduced overall 5-methyl-2′-deoxycytidine (5mdC) and reduced methylation of repetitive elements (RE), including *LINE1* and *Alu* [5]. Murine and human models suggest that global DNA hypomethylation contributes to oncogenesis by promoting chromosomal instability [6, 7].

While DNA methylation in tumors has been extensively studied, less is known about DNA methylation in normal tissues from cancer patients, or in individuals at elevated cancer risk [8]. However, DNA methylation changes have been documented in WBC from patients with breast, colorectal, bladder, and head and neck cancers [9-13]. Multiple factors appear to influence the outcome of studies of association between global DNA methylation and cancer risk, including i) sample source, i.e. blood cell type measured [14], ii) cancer type, and iii) DNA methylation analysis performed [14-16]. A recent meta-analysis found that reduced 5mdC in WBC was consistently associated with cancer, while DNA methylation changes at specific RE was not [15]. Despite these data, RE-focused studies remain attractive because of their economy and because the methylation target under study is more specifically defined. Additionally, RE are commonly hypomethylated in cancer [17], which allows investigation of associations between altered DNA methylation in cancer and normal tissues using patient-matched samples.

The *LINE1* retrotransposon comprises ∼20% of the human genome (100,000 copies/genome), is 6000-7000 bp long, and consists of a 5′ LTR, two open reading frames, and a 3′ UTR [18, 19]. Most *LINE1* elements are 5′ truncated, internally rearranged, or mutated, and have lost transposase activity [18]. In normal tissues, *LINE1* sequences are hypermethylated and located in heterochromatin, however these elements can become hypomethylated in cancer [20-25]. *LINE1* hypomethylation can also drive the expression of neighboring genes in cancer cells [26, 27]. *Alu*, a short interspersed element (SINE), is a ∼300 bp repetitive sequence and most abundant SINE, with $\sim 1 \times 10^6$ copies/genome, comprising >10% of the genome. Unlike *LINE1*, Alu sequences are CpG-rich, and contain approximately one-third of all human CpG dinucleotides [28]. While both *LINE1* and *Alu* can become hypomethylated in human cancer, the sequence context of the two elements are distinct, with *LINE1* resident in AT-rich genomic regions while *Alu* elements are resident in GC-rich regions. Thus, methylation of these two RE could be differentially regulated.

We used quantitative sodium bisulfite pyrosequencing to determine *LINE1* and *Alu* DNA methylation levels in EOC and in samples representing the normal tissue origin of EOC [29, 30]. In addition, we determined RE methylation in WBC from EOC patients, both pre- and

post-treatment, as well as in demographically-matched controls. We also compared RE methylation in tumor and WBC from matched patients. Our data provide novel insight into how RE DNA methylation is altered in EOC patients.

Materials and Methods

Patient Consent and Institutional Review Board (IRB) Approval

All study participants provided informed consent, and all human studies were approved by the Roswell Park Cancer Institute (RPCI) IRB.

Human normal ovarian surface epithelia (OSE) and fallopian tube epithelia (FTE)

Samples were collected from 14 patients undergoing bilateral salpingo-oophorectomy for non-malignant conditions (e.g. pelvic pain, history of ovarian cyst) at RPCI (Table 1). Three patients (25%) underwent risk reduction surgery based on germline *BRCA* mutations or Lynch syndrome; however, there was no evidence of occult malignancy in any patient. To obtain samples, the surface of ovaries (OSE) and distal fallopian tube fimbriae (FTE) were removed by mechanical scraping of tissues immediately following surgery, using a plastic spatula. The resulting tissues were placed into cell media, flash-frozen with liquid nitrogen, and stored at -80°C until use for genomic DNA (gDNA) extraction.

Human epithelial ovarian cancer (EOC)

41 tumor samples were collected, at the time of primary surgery prior to chemotherapy (Table 1). All EOC tissues contained at least 90% neoplastic cells.

WBC from EOC patients and controls

EOC case and control WBC were obtained from a population based case-control study named *Hormones and Ovarian Cancer Prediction* (HOPE) [31]. HOPE consists of demographic, epidemiological, and clinico-pathological data from women >25 years old diagnosed with epithelial ovarian, fallopian tube, or peritoneal cancer in Western Pennsylvania, Eastern Ohio and Western New York, as well as healthy age- and race-matched controls (Table 1). HOPE controls were free of all malignant disease, except for non-melanoma skin cancer. HOPE control and case samples consisted of buffy coats (total leukocytes), and HOPE case samples were obtained post-treatment.. The vast majority of HOPE patients received standard platinum/taxane chemotherapy (Supplemental Table 1). In addition to HOPE post-treatment samples, a group of pre-treatment samples were identified from the same population; these samples consisted of peripheral blood mononuclear cells (PBMC), and were collected after diagnosis but prior to surgery or chemotherapy. PBMC and buffy coats (total leukocytes) were prepared using standard methods, and used to isolate gDNA.

Genomic DNA (gDNA) isolation and DNA methylation analysis

gDNA extractions from EOC, OSE, and FTE were performed using the Gentra Puregene kit (Qiagen), and gDNA extractions from WBC (PBMC or leukocytes) were performed using the FlexiGene DNA kit (Qiagen). 1μg gDNA samples were sodium bisulfite-converted using the EZ DNA Methylation kit (Zymo Research). The resulting gDNA was used to determine the methylation of human *LINE1* and *Alu* using quantitative pyrosequencing, as described previously [32, 33]. Briefly, pyrosequencing was accomplished using the PSQ HS96 Pyrosequencing system (Qiagen). Non-CpG cytosines served as internal controls to verify efficient bisulfite DNA conversion, and unmethylated and methylated DNA (whole genome amplified human gDNA and M.SssI-modified human gDNA, respectively) were run as controls on each plate. Pyrosequencing was performed on duplicate samples obtained

from duplicate PCR amplifications, and assays were performed a minimum of two times. The observed methylation level of *Alu* using bisulfite pyrosequencing is artificially reduced due to conversion of methylated CpG to TpG by spontaneous deamination over evolution, but this does not impact assay performance [34].

Statistical Analyses

Statistical tests were performed using GraphPad Prism 5.0. Statistical significance was set at $P<0.05$.

Results

RE methylation in EOC and control tissues

While global DNA hypomethylation has been described in EOC, previous studies have used bulk normal ovary tissue as a control [32, 35, 36]. Because normal ovary contains stroma, immune cells, germ cells, and additional cell types that do not reflect the cell of origin of EOC, it is not an optimal control. In contrast, the surface epithelial cells of the ovary and/or the fallopian tube fimbriae are established cells of origin of EOC [29, 30]. To investigate DNA methylation specifically in these cell types, we obtained OSE and FTE from patients with non-malignant conditions, and used this material to conduct bisulfite pyrosequencing of *LINE1* and *Alu*. EOC showed robust hypomethylation as compared to OSE or FTE, while the two normal tissues were not significantly different (Fig. 1A-B; Table 2). RE methylation was also much more variable in EOC than in normal tissues (Fig. 1A-B; Table 2). RE methylation in OSE and FTE were also highly correlated (Fig. 1C). In addition, *LINE1* and *Alu* methylation were significantly correlated in EOC (Fig. 1D), confirming our earlier observations [36].

RE methylation in EOC patient and control WBC

LINE1 methylation in EOC patient (case) and control WBC is shown in Fig. 2A, and quantitative data are reported in Table 2. Interestingly, we observed significant elevation of *LINE1* methylation in cases compared to controls, but this effect was restricted to post-treatment cases (Fig 2A; Table 2). *Alu* methylation was also elevated in cases compared to controls however, for this RE, elevated methylation occurred in both pre- and post-treatment cases (Fig. 2B; Table 2). Together, the data suggest that RE methylation is elevated in EOC patient WBC. We also assessed the relationship between *LINE1* and *Alu* methylation in EOC case and control WBC. There was a statistically significant positive correlation between *LINE1* and *Alu* methylation in control leukocytes (Fig. 3A). In contrast, there was a statistically significant negative correlation between *LINE1* and *Alu* methylation in all case WBC, when considered together (Fig. 3B). In sub-group analysis, there was an indirect association between *LINE1* and *Alu* methylation in pre-treatment case PBMC, and no significant association in post-treatment case leukocytes (Fig. 3C-D).

RE methylation in patient-matched tumors and WBC

The availability of matched samples allowed us to begin to assess the relationship between EOC and normal tissue methylation in the same individuals. We restricted this comparison to tumor and pre-treatment PBMC, to eliminate the confounding effect of treatment on post-treatment leukocyte samples (and because all tumor samples were obtained before treatment). Notably, we observed no correlation of *LINE1* or *Alu* methylation between patient-matched tumors and PBMC (Fig. 4A-B).

Discussion

The goal of the current study was to provide an initial analysis of the possible utility of RE methylation as an EOC biomarker. *LINE1* and *Alu* were the focus of our study due to their high prevalence in the human genome, distinct sequence contexts, and the established methylation changes in these regions during oncogenesis. Importantly, we found that *LINE1* and *Alu* are robustly hypomethylated in EOC as compared to both OSE and FTE, the presumed tissues of origin of human EOC. OSE and FTE showed highly similar RE methylation in patient-matched samples. In contrast to our initial expectation, EOC patient WBCs were hypermethylated compared to controls. Moreover, RE tumor methylation and WBC methylation in matched EOC patients did not correlate. Taken together, the data suggest that the mechanisms controlling RE methylation in EOC patient tumors and normal tissues are distinct. In addition to *LINE1* and *Alu*, other RE, including satellite sequences, can be differentially methylated in EOC [36]. Whether methylation of other RE is similarly altered in WBC from EOC patients is worthy of future investigation.

A recent meta-analysis demonstrated no significant trend for RE element methylation changes in WBC from human cancer patients, unlike what has been observed thus far for 5mdC [15]. Consistent with this, in a previous study of human breast cancer, we observed no significant differences in *LINE1* methylation in case and control WBC [9]. However, in contrast to breast cancer, in the current study EOC case WBC showed RE hypermethylation compared to controls. While both *LINE1* and *Alu* were hypermethylated in cases, regulation at these two loci was distinct, with increases only in post-treatment cases for *LINE1*, versus both pre- and post-treatment methylation increases for *Alu*. This reinforces the idea that methylation of distinct RE may be differentially regulated. Interestingly, *LINE1* and *Alu* methylation were directly associated in control WBC, but were inversely or not associated in cases. This observation supports the notion that DNA methylation is deranged in the normal tissues of EOC patients. It is also of note that the control WBCs in our study was leukocytes, while the pre-treatment case WBCs were PBMC (pre-treatment leukocytes were not available). Because distinct global DNA methylation has been reported in different blood types [14], it is therefore possible that the methylation changes we observed between controls and pre-treatment leukocytes could be accounted for by blood cell composition.

The mechanism(s) leading to RE methylation changes in WBC (or other normal tissues) from cancer patients is currently unclear. A variety of environmental influences could impact DNA methylation and cancer risk, including toxin exposure, diet, and endocrine factors. It is also possible that treatment (surgery or chemotherapy) could impact methylation levels in WBC, and this could have influenced the results of our analysis of post-treatment cases. Further specific investigation of the impact of standard platinum/taxane chemotherapy on DNA methylation in EOC patients (if any) will be required to resolve this issue.

Although the RE methylation changes observed in WBC from EOC cases compared tocontrols were statistically significant, the control and case methylation levels over the studiedpopulations overlapped. This suggests that methylation at RE loci may have minimal utility for EOC risk assessment. This result could reflect an inherent limitation of measuring RE methylation, as it assesses methylation changes simultaneously at thousands of highly similar sequences, but positionally distinct genomic loci. In contrast to RE, Teschendorff et al. assessed DNA methylation at 27,000 CpG sites contained within single copy loci in EOC patient WBC, and discovered several candidate CpGs that showed methylation changes that may have potential utility as EOC biomarkers [37]. Thus, we believe that future work should be focused on using global methylation profiling approaches (e.g. methylome sequencing) to identify blood-based methylation biomarkers for EOC with possible diagnostic utility [38].

The observed statistically significant changes in RE methylation reported in the current study lend proof-of-principal support to such an approach. Finally, as DNA hypomethylating agents enter expanded clinical testing in EOC [39-41], the data reported here become important for fully understanding RE methylation as a pharmacodynamic measure for these novel therapeutic regimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Smitha James and Ms. Petra Link for expert technical assistance. This study was supported by NIH grants RO1CA116674 (A.R.K.), T32CA108456 (S.N.A), and R01CA126841 (KM).

References

1. Cancer Facts and Figures 2013. American Cancer Society. 2013
2. Sudo T. Molecular-targeted therapies for ovarian cancer: prospects for the future. International journal of clinical oncology. 2012; 17:424–9. [PubMed: 22915194]
3. Gopalakrishnan S, Van Emburgh BO, Robertson KD. DNA methylation in development and human disease. Mutation research. 2008; 647:30–8. [PubMed: 18778722]
4. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nature reviews Cancer. 2011; 11:726–34.
5. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene. 2002; 21:5400–13. [PubMed: 12154403]
6. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003; 300:455. [PubMed: 12702868]
7. Karpf AR, Matsui S. Genetic disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. Cancer research. 2005; 65:8635–9. [PubMed: 16204030]
8. Marsit C, Christensen B. Blood-derived DNA methylation markers of cancer risk. Advances in experimental medicine and biology. 2013; 754:233–52. [PubMed: 22956505]
9. Choi JY, James SR, Link PA, McCann SE, Hong CC, Davis W, et al. Association between global DNA hypomethylation in leukocytes and risk of breast cancer. Carcinogenesis. 2009; 30:1889–97. [PubMed: 19584139]
10. Hsiung DT, Marsit CJ, Houseman EA, Eddy K, Furniss CS, McClean MD, et al. Global DNA methylation level in whole blood as a biomarker in head and neck squamous cell carcinoma. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007; 16:108–14.
11. Lim U, Flood A, Choi SW, Albanes D, Cross AJ, Schatzkin A, et al. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. Gastroenterology. 2008; 134:47–55. [PubMed: 18166347]
12. Moore LE, Pfeiffer RM, Poscablo C, Real FX, Kogevinas M, Silverman D, et al. Genomic DNA hypomethylation as a biomarker for bladder cancer susceptibility in the Spanish Bladder Cancer Study: a case-control study. The lancet oncology. 2008; 9:359–66. [PubMed: 18339581]
13. Pufulete M, Al-Ghnaniem R, Leather AJ, Appleby P, Gout S, Terry C, et al. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology. 2003; 124:1240–8. [PubMed: 12730865]
14. Wu HC, Delgado-Cruzata L, Flom JD, Kappil M, Ferris JS, Liao Y, et al. Global methylation profiles in DNA from different blood cell types. Epigenetics : official journal of the DNA Methylation Society. 2011; 6:76–85. [PubMed: 20890131]

15. Brennan K, Flanagan JM. Is there a link between genome-wide hypomethylation in blood and cancer risk? Cancer Prev Res (Phila). 2012; 5:1345–57. [PubMed: 23135621]
16. Woo HD, Kim J. Global DNA hypomethylation in peripheral blood leukocytes as a biomarker for cancer risk: a meta-analysis. PloS one. 2012; 7:e34615. [PubMed: 22509334]
17. Karpf AR. Genome-wide hypomethylation and cancer risk--letter. Cancer Prev Res (Phila). 2013; 6:753. [PubMed: 23752487]
18. Fanning TG, Singer MF. LINE-1: a mammalian transposable element. Biochimica et biophysica acta. 1987; 910:203–12. [PubMed: 2445384]
19. Pickeral OK, Makalowski W, Boguski MS, Boeke JD. Frequent human genomic DNA transduction driven by LINE-1 retrotransposition. Genome research. 2000; 10:411–5. [PubMed: 10779482]
20. Jurgens B, Schmitz-Drager BJ, Schulz WA. Hypomethylation of L1 LINE sequences prevailing in human urothelial carcinoma. Cancer research. 1996; 56:5698–703. [PubMed: 8971178]
21. Kazazian HH Jr, Moran JV. The impact of L1 retrotransposons on the human genome. Nature genetics. 1998; 19:19–24. [PubMed: 9590283]
22. Suter CM, Martin DI, Ward RL. Hypomethylation of L1 retrotransposons in colorectal cancer and adjacent normal tissue. International journal of colorectal disease. 2004; 19:95–101. [PubMed: 14534800]
23. Takai D, Yagi Y, Habib N, Sugimura T, Ushijima T. Hypomethylation of LINE1 retrotransposon in human hepatocellular carcinomas, but not in surrounding liver cirrhosis. Jpn J Clin Oncol. 2000; 30:306–9. [PubMed: 11007163]
24. Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thong-ngam D, et al. Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. Oncogene. 2004; 23:8841–6. [PubMed: 15480421]
25. Florl AR, Lower R, Schmitz-Drager BJ, Schulz WA. DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. British journal of cancer. 1999; 80:1312–21. [PubMed: 10424731]
26. Wolff EM, Byun HM, Han HF, Sharma S, Nichols PW, Siegmund KD, et al. Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. PLoS genetics. 2010; 6:e1000917. [PubMed: 20421991]
27. Hur K, Cejas P, Feliu J, Moreno-Rubio J, Burgos E, Boland CR, et al. Hypomethylation of long interspersed nuclear element-1 (LINE-1) leads to activation of proto-oncogenes in human colorectal cancer metastasis. Gut. 2013
28. Batzer MA, Deininger PL. Alu repeats and human genomic diversity. Nature reviews Genetics. 2002; 3:370–9.
29. Karst AM, Drapkin R. Ovarian cancer pathogenesis: a model in evolution. J Oncol. 2010; 2010:932371. [PubMed: 19746182]
30. Dubeau L. The cell of origin of ovarian epithelial tumours. The lancet oncology. 2008; 9:1191–7. [PubMed: 19038766]
31. Kurta ML, Moysich KB, Weissfeld JL, Youk AO, Bunker CH, Edwards RP, et al. Use of fertility drugs and risk of ovarian cancer: results from a U.S.-based case-control study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2012; 21:1282–92.
32. Woloszynska-Read A, Mhawech-Fauceglia P, Yu J, Odunsi K, Karpf AR. Intertumor and intratumor NY-ESO-1 expression heterogeneity is associated with promoter-specific and global DNA methylation status in ovarian cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008; 14:3283–90. [PubMed: 18519754]
33. Bollati V, Fabris S, Pegoraro V, Ronchetti D, Mosca L, Deliliers GL, et al. Differential repetitive DNA methylation in multiple myeloma molecular subgroups. Carcinogenesis. 2009; 30:1330–5. [PubMed: 19531770]
34. Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic acids research. 2004; 32:e38. [PubMed: 14973332]

35. Widschwendter M, Jiang G, Woods C, Muller HM, Fiegl H, Goebel G, et al. DNA hypomethylation and ovarian cancer biology. Cancer research. 2004; 64:4472–80. [PubMed: 15231656]

36. Woloszynska-Read A, Zhang W, Yu J, Link PA, Mhawech-Fauceglia P, Collamat G, et al. Coordinated cancer germline antigen promoter and global DNA hypomethylation in ovarian cancer: association with the BORIS/CTCF expression ratio and advanced stage. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011; 17:2170–80. [PubMed: 21296871]

37. Teschendorff AE, Menon U, Gentry-Maharaj A, Ramus SJ, Gayther SA, Apostolidou S, et al. An epigenetic signature in peripheral blood predicts active ovarian cancer. PloS one. 2009; 4:e8274. [PubMed: 20019873]

38. Nagarajan RP, Fouse SD, Bell RJ, Costello JF. Methods for cancer epigenome analysis. Advances in experimental medicine and biology. 2013; 754:313–38. [PubMed: 22956508]

39. Fang F, Balch C, Schilder J, Breen T, Zhang S, Shen C, et al. A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. Cancer. 2010; 116:4043–53. [PubMed: 20564122]

40. Matei D, Fang F, Shen C, Schilder J, Arnold A, Zeng Y, et al. Epigenetic resensitization to platinum in ovarian cancer. Cancer research. 2012; 72:2197–205. [PubMed: 22549947]

41. Odunsi K, Matsuzaki J, James SR, Mhawech-Fauceglia P, Tsuji T, Miller A, et al. Epigenetic Potentiation of NY-ESO-1 Vaccine Therapy in Human Ovarian Cancer. Cancer Immunology Research. 2014; 2

Highlights

- EOC displayed repetitive element (RE) DNA hypomethylation compared to normal surface ovarian or fallopian tube epithelia
- WBC from EOC patients displayed RE DNA hypermethylation compared to controls
- RE DNA methylation in EOC patient-matched tumors and WBC did not correlate

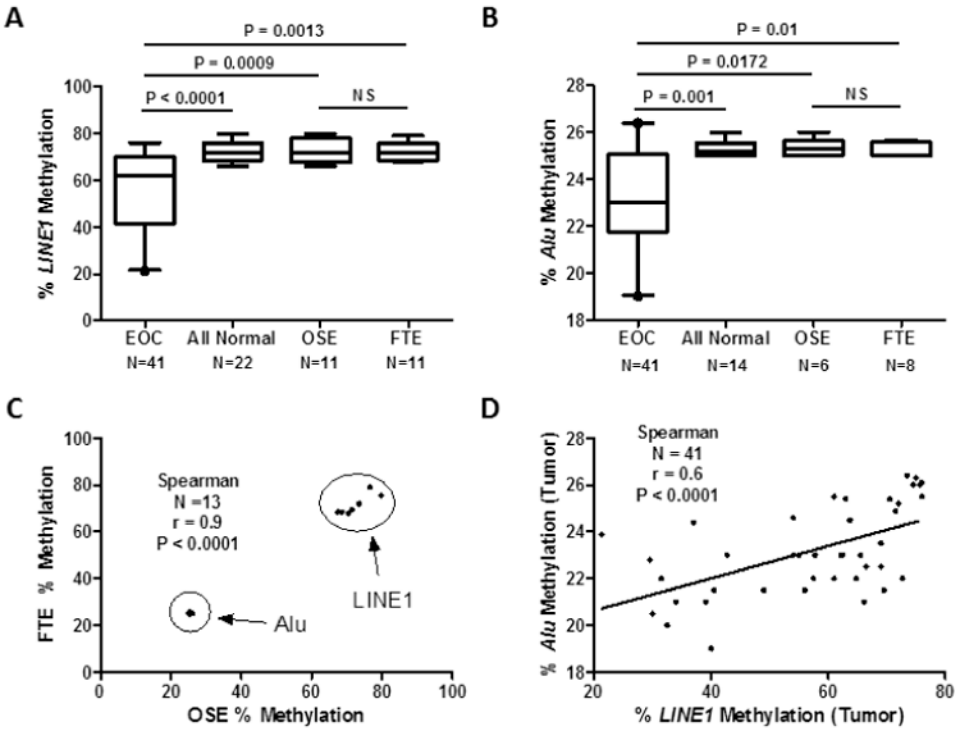

Figure 1. *LINE1* and *Alu* RE methylation in human epithelial ovarian cancer (EOC), normal ovarian surface epithelia (OSE), and normal fallopian tube epithelia (FTE)

LINE1 and *Alu* methylation were determined using bisulfite pyrosequencing. **A**) *LINE1* and **B**) *Alu* methylation in EOC, normal tissues (OSE + FTE), OSE, and FTE samples. Number of samples per group is shown below the y-axis labels. Box and whiskers plots are shown (bars, mean; box, 25-75 percentile; whiskers, 2.5-97.5 percentile). Mann-Whitney two-tailed P-values are shown. NS = not significant. **C**) Comparison of methylation in patient-matched OSE and FTE tissues. Spearman correlation test results (for *LINE1* + *Alu*) are shown. **D**) Comparison of *LINE1* and *Alu* methylation in EOC. A linear regression line, and Spearman correlation test results are shown.

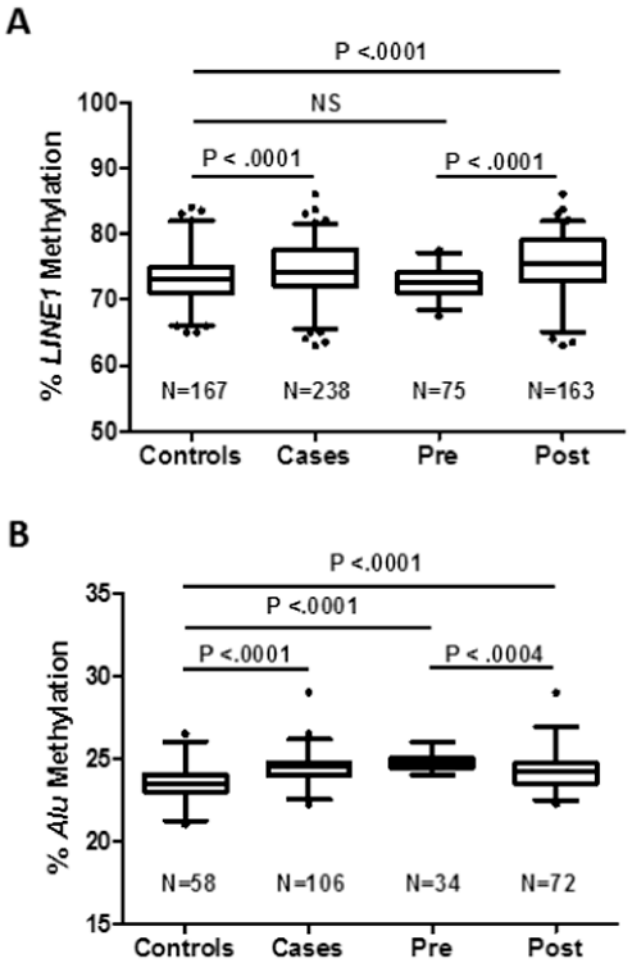

Figure 2. *LINE1* and *Alu* RE methylation in WBC from EOC patients and controls

RE methylation was determined using bisulfite pyrosequencing. Post-treatment case and control leukocytes were obtained from the HOPE study. Pre-treatment case samples (PBMC) were also available, for a sub-set of HOPE cases. **A**) *LINE1* methylation in controls (leukocytes), all cases (PBMC + leukocytes), pre-treatment cases (PBMC), and post-treatment cases (leukocytes). **B**) *Alu* methylation in controls (leukocytes), all cases (PBMC + leukocytes), pre-treatment cases (PBMC), and post-treatment cases (leukocytes). Box and whiskers plots are presented as described in Fig. 1. Number of samples per group is shown below the y-axis labels. Mann-Whitney two-tailed P-values are shown.

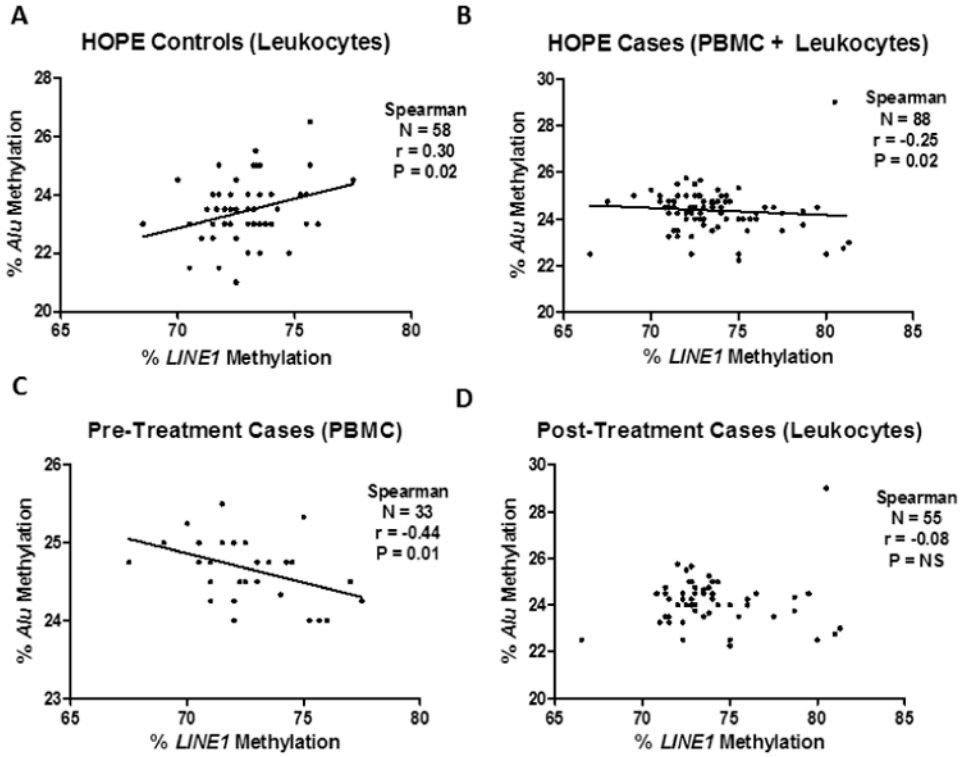

Figure 3. Correlation of *LINE1* and *Alu* methylation in WBC from EOC patients and controls

RE methylation was determined using bisulfite pyrosequencing. **A**) Association between *LINE1* and *Alu* methylation in controls (leukocytes). **B**) Association between *LINE1* and *Alu* methylation in all cases (PBMC + leukocytes). **C** Association between *LINE1* and *Alu* methylation in pre-treatment cases (PBMC). **D**) Association between *LINE1* and *Alu* methylation in post-treatment cases (leukocytes). In all panels, Spearman correlation test results are shown, and linear regression lines are plotted for statistically significant correlations. NS = not significant.

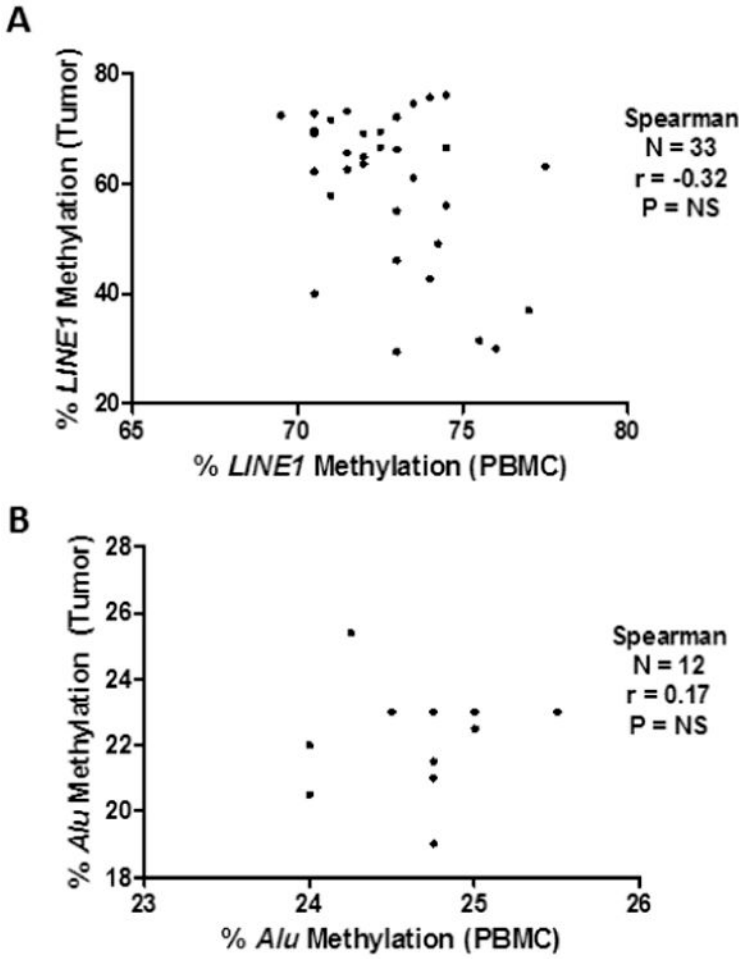

Figure 4. Correlation of RE methylation in patient-matched tumors and pre-treatment PBMC

RE methylation was determined using bisulfite pyrosequencing. **A**) *LINE1* and **B**) *Alu* methylation were compared in patient-matched tumors and pre-treatment PBMC samples. Spearman correlation test results are shown. NS = not significant.

Table 1

Study Populations

Sample Category	Descriptor	Value
OSE[1]	N	14
	Age (mean)	49.4
	Age (range)	33-64
FTE[2]	N	12
	Age (mean)	47.4
	Age (range)	33-64
EOC[3]	N	41
	Age (mean)	61
	Age (range)	37-89
	Tumor Grade	
	1-2	8 (19.5%)
	3	33 (80.5%)
	Tumor Stage	
	1	4 (9.8%)
	2	5 (12.2%)
	3	27 (65.9%)
	4	5 (12.2%)
	Tumor Histology	
	Clear Cell	4 (9.8%)
	Endometriod	1 (2.4%)
	Mucinous	3 (7.3%)
	Serous	27 (65.9%)
	Mixed	5 (12.2%)
	Transitional Cell	1 (2.4%)
HOPE Controls[4]	N	167
	Age (mean)	58.1
	Age (range)	34-83
	Race	
	Caucasian	158 (94.6%)
	Other	9 (5.3%)
HOPE Cases[5]	N	181
	Age (mean)	60.4
	Age (range)	27-89
	Race	
	Caucasian	167 (92.3%)

Sample Category	Descriptor	Value
	Other	14 (7.7%)

[1] Normal human ovarian surface epithelium

[2] Normal human fallopian tube epithelium

[3] Human epithelial ovarian cancer

[4] Hormones and Ovarian Cancer Prediction study (Controls)

[5] Hormones and Ovarian Cancer Prediction study (Cases)

Table 2

Summary of DNA Methylation data[1]

Sample Category	*LINE1* % Methylation				*Alu* % Methylation			
	N	Mean	SD	Range	N	Mean	SD	Range
OSE[2]	11	72.5	5.0	66-79.8	6	25.4	0.4	25-26
FTE[3]	11	72.3	3.5	68-79	8	25.2	0.3	25-25.7
EOC[4]	41	57.1	15.7	21.3-76	41	23.2	1.9	19-26.4
HOPE Controls[5]	167	73.1	3.8	65-84	58	23.4	1.0	21-26.5
HOPE Cases[6]	238	74.6	4.0	63-86	106	24.4	0.9	22.3-29
HOPE Pre-Treatment Cases[7]	75	72.5	2.1	67.5-77.5	34	24.7	0.5	24-26
HOPE Post-TreatmentCases[8]	163	75.5	4.3	63-86	72	24.2	1.0	22.3-29

[1] DNA methylation determined using quantitative sodium bisulfite pyrosequencing

[2] Normal human ovarian surface epithelia

[3] Normal human fallopian tube epithelia

[4] Human epithelial ovarian cancers (tumors)

[5] Hormones and Ovarian Cancer Prediction Study (Controls: Leukocytes)

[6] Hormones and Ovarian Cancer Prediction Study (Cases: PBMC + Leukocytes)

[7] Peripheral blood mononuclear cells (PBMC)

[8] Leukocytes

Gynecol Oncol. Author manuscript; available in PMC 2015 February 01.