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

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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.

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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 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,

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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 were 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 ~1×10^6 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 is 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-treatments, 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.

Table 1
Study populations.

Sample category	Descriptor	Value
OSE[a]	N	14
	Age (mean)	49.4
	Age (range)	33–64
FTE[b]	N	12
	Age (mean)	47.4
	Age (range)	33–64
EOC[c]	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[d]	N	167
	Age (mean)	58.1
	Age (range)	34–83
	Race	
	Caucasian	158 (94.6%)
	Other	9 (5.3%)
HOPE cases[e]	N	181
	Age (mean)	60.4
	Age (range)	27–89
	Race	
	Caucasian	167 (92.3%)
	Other	14 (7.7%)

[a] Normal human ovarian surface epithelium.
[b] Normal human fallopian tube epithelium.
[c] Human epithelial ovarian cancer.
[d] Hormones and ovarian cancer prediction study (controls).
[e] Hormones and ovarian cancer prediction study (cases).

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