Gynecologic Oncology 131 (2013) 198–206

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Error-promoting DNA synthesis in ovarian cancer cells

Heqiao Dai [a], Robert J. Hickey [b], Jianying Liu [c], Robert M. Bigsby [d], Carita Lanner [c,1], Linda H. Malkas [e,*]

[a] Indiana University School of Medicine, Department of Medicine, Division of Clinical Pharmacology, USA
[b] City of Hope Beckman Research Institute, Department of Molecular Pharmacology, USA
[c] Indiana University School of Medicine, Department of Medicine, USA
[d] Indiana University School of Medicine, Department of Obstetrics and Gynecology, USA
[e] City of Hope Beckman Research Institute, Department of Molecular and Cellular Biology, USA

HIGHLIGHTS

- The DNA replication apparatus of ovarian cancers has a DNA synthesis process that is error-promoting.
- There is a DNA polymerase efficiency difference between non-malignant and ovarian cancer cells for specific types of nucleotide mismatches.
- Systematic analysis of ovarian cancer DNA replication could uncover mechanisms that drive genetic damage accumulation and contribute to disease.

ARTICLE INFO

Article history:
Received 19 December 2012
Accepted 14 June 2013
Available online 12 July 2013

Keywords:
Ovarian cancer
DNA replication
Multiprotein complex
Replication fidelity

ABSTRACT

Objective. The objective of this study is to determine whether an altered DNA replication process is responsible for some of genetic damage observed in ovarian cancer.

Methods. The replication fidelity of the DNA synthetic process was evaluated in both malignant and non-malignant human ovarian cells. The types of replication errors produced were identified. In addition, kinetic analyses of the efficiency of ovarian cancer DNA polymerases for misincorporating nucleotides were performed.

Results. We report for the first time that ovarian cancer cells harbor an error promoting DNA replication apparatus which contributes to the decrease in DNA synthetic fidelity exhibited by these cells. Our study also shows that the decrease in DNA replication fidelity was not a result of an increased DNA replication activity. In addition, it was observed that the higher rate of DNA replication errors does not result in significant differences in the type of DNA replication-errors made during the DNA replication process; just the relative abundance. A detailed kinetic analysis of the efficiency of misincorporating nucleotides demonstrated that the DNA polymerases within the ovarian cancer cells exhibited a significant propensity for creating purine–pyrimidine nucleotide mismatches relative to non-malignant ovarian cells, while being only slightly more efficient at incorrectly pairing a purine nucleotide with a purine nucleotide.

Conclusions. All together, these data suggest that the systematic analysis of the DNA replication process in ovarian cancer could uncover information on some of the molecular mechanisms that drive the accumulation of genetic damage, and probably contribute to the pathogenesis of the disease.

Introduction

Genetic instability is expressed in cancer in diverse ways [1]. It may affect DNA replication associated nucleotide proofreading (leading to base substitutions, deletions, or additions), chromosomal structure (producing translocations, sequence gains, or losses), or karyotypic integrity (resulting in aneuploidy). None-the-less, it is very likely that genetic instability either induces or accelerates the proliferation of cancer cells by favoring the emergence of variant cells. It has already been shown that a controlled alteration of genes involved in genome maintenance promotes or favors carcinogenesis [2,3].

In ovarian tumors there is a strong correlation between high rates of DNA synthesis and poor over-all prognosis [4–10]. Based on the available data, it appears that high levels of ovarian cancer cell DNA synthesis are associated with an increased probability of lymph-node metastases [11,12]. Increasingly, data have showed that large numbers of mutations present in a variety of human malignancies originate from both DNA synthesis errors and DNA repair deficits [1,13]. The malignant cell phenotype appears as the result of a multi-step development process that is associated with the accumulation of multiple genetic mutations. The

* Corresponding author at: City of Hope Beckman Research Institute, 1500 East Duarte Road, Duarte, CA 91010, USA. Fax: +1 626 471 3602.
E-mail address: lmalkas@coh.org (L.H. Malkas).
[1] Presently at Northern Ontario School of Medicine, Division of Medical Sciences, USA.

0090-8258
http://dx.doi.org/10.1016/j.ygyno.2013.06.022

fidelity of DNA replication is thought to have a pivotal role in regulating the occurrence and development of cancer via a multistage carcinogenic transformation [1,13]. The observation that there is extensive genetic damage in ovarian cancer cells has been associated with a higher than normal rate of DNA synthesis. Therefore, the high rates of DNA synthesis and extensive genetic damage exhibited by ovarian tumors suggest that an alteration in the DNA replication machinery of these cells could result in uncontrolled and error-promoting DNA synthesis.

In an effort to elucidate the role of DNA replication in cancer cell biology, we have isolated and purified from non-malignant and malignant human cell lines and tissues an assembly of DNA replication proteins, collectively termed the DNA synthesome, that have been shown to execute in vitro DNA replication [14–19]. The mammalian cell DNA replication complex is an organized structure that is fully competent to orchestrate, in vitro, the reactions required to efficiently replicate DNA in intact cells using the well known simian virus 40 (SV40) replication system [14,19]. The human cell DNA synthesome has been extensively purified using a combination of biochemical steps that include centrifugation, polyethylene glycol precipitation, Q-Sepharose column chromatography, velocity sedimentation, and native PAGE. The DNA synthesome complex has been shown to contain all the essential proteins necessary to carry out in vitro DNA replication; DNA polymerases (pols) α, δ, and ε, proliferating cell nuclear antigen (PCNA), replication factor A (RPA), replication factor C (RFC), topoisomerases I and II, and DNA ligase I [14–19].

In this article is a description of the results of an evaluation of the DNA synthesome of ovarian cancer cells. We report here for the first time that ovarian cancer cells harbor a mutagenic DNA replication apparatus. We anticipate that further examination of the DNA replication apparatus of ovarian cancer cells is likely to uncover altered protein factors that play a critical role in the process leading to the accumulation of some of the genetic damage, and that these altered factors may themselves become a hallmark of this disease. In addition, since genetic damage appears to be a cumulative process in ovarian cancer, it stands to reason that alteration of DNA replication proteins may be an early event in the development of this cancer. Therefore, the systematic analysis of the DNA replication process in ovarian cancer could uncover information on some of the molecular mechanisms that drive the accumulation of genetic damage, and probably contribute to the pathogenesis of the disease. It is our contention that a better understanding of the pathogenesis of ovarian cancer, (i.e. by describing key molecular events in the development and maintenance of the disease), may facilitate the discovery of improved strategies for the prevention, detection and treatment of ovarian carcinoma.

Materials and methods

Primary non-malignant ovarian surface epithelial (serosa) cell cultures

Non-malignant primary ovarian surface epithelial (NOSE-1 and NOSE-2) cells were prepared from two different individuals. These cells were cultured from a "lateral slice" taken from overtly normal ovaries acquired during surgery on non-malignant gynecological patients with no history of receiving chemotherapy (as described in 20–22). The slice was cultured in a 60-mm dish, epithelial side down, in a layer of medium to prevent the drying of tissue and to nourish the cells. The medium was MCDB105/M199 (50:50), 15% FBS, rhEGF (1 μg/ml), 200 mM glutamine, 100 mM sodium pyruvate, gentamycin (25 μg/ml), penicillin (10 U/ml), and streptomycin (10 μg/ml). As cells grew out from the tissue specimen, the tissue was moved to another site in the dish until the dish was 50% confluent. At 80–90% confluency, the cells were trypsinized and transferred to a 100-mm dish and a multiwell slide. The slide was used to check cells for the co-expression of vimentin and cytokeratin, using immunocytochemistry. All cultures were shown to be 100% positive for both markers. Cell cultures were expanded by passaging 4–5 times before harvesting cell pellets and freezing the pellets at −80 °C. DNA synthesome was prepared from the cell pellets made individually from NOSE-1 and NOSE-2 as described below.

Ovarian cancer cell cultures

Three different cisplatin sensitive ovarian cancer cell lines were used in the studies. Hey C2 and OVCAR 432 cells were cultured as monolayers in Minimum Essential Medium (MEM) (GIBCO-BRL) supplemented with 10% fetal bovine serum (FBS), 10 units/ml penicillin and 10 μg/ml streptomycin. 10 μM sodium pyruvate was added to the medium during the culture of the OVCAR 432 cells. Hey C2 is a papillary cystadenocarcinoma cell line [23]. OVCAR-432 is an ovarian carcinoma (histological type unknown) cell line [24]. BG-1 cells were cultured in Dulbecco's Modified Eagle's Medium with Ham's supplement F12 (DMEM/F12) (Sigma), 5% FBS, and 10 mg/ml kanamycin. BG-1 is derived from a poorly differentiated ovarian adenocarcinoma [25]. When cultures had proliferated approximately 80% confluency, the cells were scraped into cold phosphate-buffered saline (PBS) solution and collected by centrifugation at 1000 rpm, 5 min at 4 °C. The cells were re-suspended and washed three times with PBS, and then pelleted before being stored at −80 °C.

Purification scheme for the ovarian cell DNA synthesome from cell cultures

DNA synthesome preparations were prepared from ovarian cell lines grown in culture as described above. Cells were harvested and cell pellets were stored at −80 °C until used for synthesome preparation. For synthesome preparation, the cell pellets were thawed on ice, homogenized, fractionated and purification of the synthesome was performed in a series of centrifugation steps and resolution through a Q-Sepharose chromatography column as previously described [14,19]. The protein concentrations of the synthesome fractions were measured using the Bradford method. DNA synthesome preparations were stored in aliquots at −80 °C until used for analyses.

In vitro DNA replication assay

The activity of the DNA synthesome to support SV40 in vitro DNA replication in the presence of viral large T-antigen was performed as described previously [14,19]. One unit of DNA replication activity was designated as the incorporation of 1 pmol of radiolabeled deoxynucleotide into DNA at 37 °C per time indicated in the text.

DNA replication fidelity assay

The DNA from each in vitro DNA replication reaction was precipitated and subjected to DpnI digestion as described previously [26,27]. The DpnI-digested in vitro replicated DNA was used to transfect the *Escherichia coli* host as described previously [26,27]. The transfection and plating conditions give intense blue color for the wild-type plasmid, which facilitates the visualization of mutant phenotypes. The mutant colonies range from white to intermediate (relatively blue) phenotypes.

Synthesome based primer extension assay

Both the 18 nucleotide primer (P), containing a 5′ fluorescence tag, and each of the 36 nucleotide templates (T) were synthesized by MCR Inc. (a Midland Certified Reagent Company). The P/T single stranded DNA's (ssDNA) were annealed to one another using a ratio of 1 primer to 1.2 templates by heating the combined primers and templates to 90 °C in freshly deionized water, followed by gradually cooling the mixture to room temperature. T4-phage DNA polymerase was purchased from US Biochemical Corporation, and used to generate full-length DNA in control primer extension assays. Ten-microliter reaction mixtures containing 2 pM primer/template DNA, 20 μg DNA synthesome fraction, 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM DTT, 0.5 mg/ml BSA, 10% glycerol, and different concentrations of deoxynucleotide tri-phosphates

(dNTP) (as specified in the text), were incubated at 37 °C for 60 min. The reaction products were resolved by electrophoresis through a 15% polyacrylamide gel containing 7 M Urea in Tris–Borate–EDTA buffer (TBE) after quenching the reaction by adding 5 μl of 99.9% formamide. The polyacrylamide gel was scanned using a Fujinon imager (LAS-3000IR) and the fluorescence intensity of individual bands in the gel was quantified by Fluorescent Image Scanning analytical software (FMBIO II). The frequency of incorporating the non-complementary nucleotide (i.e., the frequency of misincorporation [which is a measure of the fidelity of the reaction giving rise to the 36 nt gel band]), was determined as the ratio of the fluorescence intensity of the 36 nt band of DNA containing the misincorporated nucleotide, to the fluorescence intensity of the 26 nt band of DNA containing no nucleotide misincorporations. Eq. I: $\mathbf{f\ misin} = \mathbf{I}_{\sum(T+1)} / \mathbf{I}_{\sum(T-1)}$ was used to calculate the frequency of misincorporation, where $(\mathbf{I}_{\sum(T+1)} = \mathbf{I}_{T+1} + \mathbf{I}_{T+2} + \ldots\ldots\ \mathbf{I}_{T+9}$, and $\mathbf{I}_{\sum(T-1)} = \mathbf{I}_{T} + \mathbf{I}_{T-1} + \mathbf{I}_{T-2} + \ldots\ldots\ \mathbf{I}_{T-8})$. The frequency of nucleotide misincorporation was plotted as a function of the concentration of the non-complementary nucleotide incorporated into the reaction product, which was used to perform a Michaelis–Menten kinetic analysis of the frequency of nucleotide misincorporation supported by the DNA synthesome from the ovarian cancer cells. V_{max} (maximal velocity) and K_m (concentration of substrate at half-maximal activity) were determined using GraphPad IV (Prism) software. The Efficiency (E) of nucleotide misincorporation was determined as the ratio of V_{max} to K_m using Eq. II: $E_{misin} = V_{max} / K_m$ [28]. All kinetic values were statistically analyzed and compared using a statistical method of the *t*-test to determine the difference of nucleotide misincorporation by the ovarian cancer cells and the NOSE cells.

Results

Ovarian cancer cells harbor a mutagenic DNA replication apparatus

To assess the replication fidelity of the synthesome isolated from malignant and non-malignant human ovarian cells the DNA synthesome replication complex was prepared from the malignant ovarian cell lines Hey C2, BG-1, and OVCAR-432. For the purpose of comparison, two different non-malignant primary ovarian cell lines (NOSE-1 and -2), prepared from two different individuals were used. The synthesome was isolated from these cells as described in the Materials and methods section. The DNA replication fidelity of these preparations was evaluated using a forward mutagenesis assay as described in the Materials and methods section. The resulting data as described in Fig. 1 show that the synthesome fraction derived from ovarian cancer cells produced significantly more nucleotide errors in nascent DNA than did the complex from non-malignant cells (~2.5 to almost 5.0-fold). These data suggest that a distinctly error-promoting replication apparatus was a significant characteristic of the malignant human ovarian cells evaluated.

Error-promoting DNA replication of malignant ovarian cells does not correlate with an increased rate of in vitro DNA synthesis

To validate that the observed increase in the mutation frequency of the purified malignant ovarian cell replication apparatus was not merely due to an increase in the rate of in vitro DNA synthesis, the amount of nascent DNA formed during the DNA replication assay mediated by the DNA synthesome derived from malignant and non-malignant ovarian cells was examined. The replication activity of the DNA synthesome preparations was examined using the in vitro SV40 DNA replication assay described in the Materials and methods section. The incorporation of ([α^{32}P]-dCMP) into the nascent daughter DNA molecules was measured and the level of replication activity was expressed as picomole nascent DNA synthesized per microgram of DNA synthesome protein per hour [Table 1A]. It was observed that the large T-antigen dependent replication activity of the malignant ovarian DNA synthesome was not significantly higher than that of the non-malignant ovarian complex [Table 1A]. These data indicate that the significant decrease in DNA replicative fidelity observed for the malignant ovarian cell DNA synthesome [Fig. 1] was not related to an increased in vitro DNA replication activity exhibited by the replication complex.

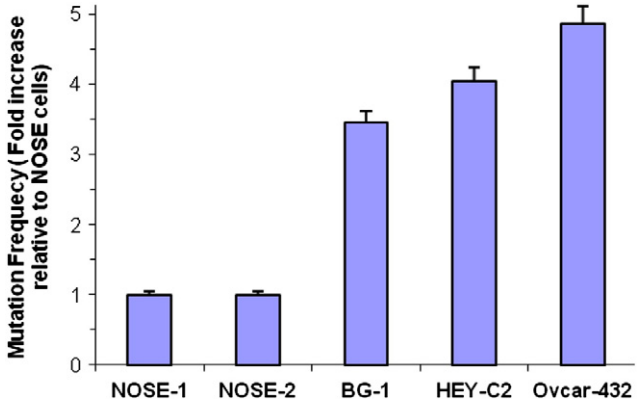

Fig. 1. Error-promoting DNA synthesis levels for DNA synthesome mediated DNA replication in malignant and non-malignant ovarian cancer cell extracts. The levels of error-promoting synthesis obtained during in vitro DNA replication mediated by the DNA synthesome isolated from malignant and non-malignant ovarian cell extracts are represented. The error frequency is represented as a ratio of the frequency of synthetic errors made by the malignant cell synthesome fraction versus that made by the NOSE-1 and NOSE-2 cell derived synthesome fractions.

The types of DNA replication errors made by malignant and non-malignant ovarian cell synthesome

In order to determine the types of mutations prevalent in the nascent DNA produced by the synthesome of malignant and non-malignant ovarian cells, the target gene (*lacZα*) contained in the in vitro DNA replication product was sequenced as described in [26]. The in vitro replicated plasmid DNA present in mutant (white phenotype) bacterial transformants produced during the forward mutagenesis assay was extracted, purified, and sequenced. To serve as a positive control, plasmid DNA isolated from wild-type (dark blue) transformants was purified, sequenced, and compared to the published sequence of the *lacZα* gene (Stratagene). These wildtype *lacZα* sequences were used to identify the nucleotide sequence errors within the target gene sequence. Automated dideoxynucleotide sequencing of the double-stranded *lacZα* gene demonstrated that, while the number of mutations present in

Table 1
(A) Rate of DNA synthesis mediated by the DNA synthesome prepared from malignant and non-malignant ovarian epithelial cells. (B) Frequency of specific types of replication errors produced by the DNA synthesome derived from non-malignant and malignant ovarian cells.

(A)

Source of DNA synthesome	T-antigen dependent in vitro DNA replication (units)[a]	Fold T-antigen dependent in vitro DNA replication
Non-malignant NOSE-1	17.2	0.96
Non-malignant NOSE-2	18.8	1.04
Hey C2	19.2	1.06
BG-1	18.3	1.01
OVCAR-432	16.9	0.94

(B)

Mutation type	Malignant cells	Non-malignant cells
Mismatch	88.1	87.5
Deletion	5.8	6.2
Insertion	6.1	6.3
All	100.0	100.0

(% of total mutations observed.)
[a] Units = picomoles nascent DNA synthesized per microgram of DNA synthesome protein per hour.

the DNA replication products created by the malignant ovarian cell synthesome derived from BG-1, HEY-C2 and OVACAR-432 cells was greater than those formed by the non-malignant complex derived from NOSE-1 and NOSE-2 cells [Fig. 1], there was no significant difference in the types of mutations found in the replicated DNA products [Table 1B]. It was also observed that there were neither mutational hot spots, nor any particular type of mismatch that was found to occur more frequently than another in the replicated *lacZα* gene.

Detailed kinetic analyses of the altered replication fidelity and nucleotide misincorporation in ovarian cancer cells

As a step toward understanding the molecular mechanism(s) mediating the reduction in DNA synthetic fidelity exhibited by ovarian cancer cells relative to non-malignant ovarian surface epithelial cells, we used the method described by Creighton and Goodman [28] to compare the kinetics of nucleotide incorporation into nascent DNA by DNA synthesome fractions isolated from these cells. Using DNA synthesome preparations from two non-malignant ovarian surface epithelial cell lines (NOSE-1 and -2) and three established ovarian cancer cell lines (BG-1, Hey C2, OVCAR-432); we quantified the extent to which non-complementary nucleotides were incorporated into newly synthesized DNA, as described in the Materials and methods section. Nascent DNA produced in a DNA polymerase extension assay used a primed single-stranded DNA template, [shown in Fig. 2A], and the reaction products were resolved from one another by polyacrylamide gel electrophoresis [Materials and methods]. Lanes 1–3 are the control/reference lanes, identifying the position of the 3 most abundant reaction products. Lane 1 contains only dialyzed synthesome fraction and the 18 nucleotide (nt) long primed DNA template; 0nly the position of the 18 nt long primer is visible. The reaction products formed by purified T4 bacteria phage DNA polymerase, in the presence of the primed DNA template and the reaction mixture are shown in lanes 2–3. The reaction mixture contains, either dATP, as the sole nucleotide triphosphate (lane 2), which supports synthesis of the nascent (26 nt) DNA up to the dCMP in the template strand (i.e., 26 nt in length), or dATP and dGTP, which support synthesis of the full-length (36 nt) nascent DNA (lane 3). Both the source of the DNA synthesome fraction (cell line) and the precise nucleotide concentration used in a specific reaction mixture are indicated at the bottom of each lane of the gel. The extent of the DNA synthetic reaction supported by each reaction mixture and DNA synthesome fraction was determined by quantifying the fluorescence intensity of the 26 nt and 36 nt reaction products (Materials and methods). We reasoned that if the synthesome fraction supports a high degree of fidelity, as would be expected for the synthesome isolated from the NOSE cells, the predominant product formed would be only 26 nt in length with dATP as the sole nucleotide in the reaction mixture. However, if the fidelity of the synthesome fraction is reduced relative to that of the synthesome fraction from the NOSE cells, we expect the 36 nt full-length DNA product to accumulate. This expectation was based upon the requirement that dATP must be misincorporated into the nascent DNA strand, opposite the dCMP in the template; prior to continuing to extend the newly synthesized DNA. We also anticipated that the frequency of misincorporation would be proportional to the concentration of the dATP.

The data shown in Fig. 2B confirmed our expectations, and demonstrated that the frequency of misincorporation of dATP into the 36 nt strand, was directly proportional to the concentration of the nucleotide triphosphate, and that misincorporation of dATP occurred more frequently with synthesome from ovarian cancer cells, than from the NOSE cells. The DNA polymerase activity in the synthesome fraction from BG-1 cells exhibits a higher degree of DNA synthetic fidelity during the elongation of the primed DNA template than that of the corresponding fractions from OVCAR-432 and Hey C2 cells. In Fig. 2C, the data indicated that misincorporation of dATP opposite the dCMP in the primed template was directly proportional to the concentration of dATP in the reaction mixture. Interestingly, while the frequency of misincorporation of dATP into nascent DNA varied considerably between individual cancer cell lines (Table 2), and increased in direct proportion to the nucleotide concentration in the reaction mixture (Fig. 2C); misincorporation of dATP into newly synthesized DNA by the NOSE cell synthesome fraction was quite low, and remained essentially constant; regardless of the concentration of dATP in the reaction mixture (Fig. 2B). These data suggested that the fidelity with which NOSE cell DNA polymerase incorporated nucleotides into nascent DNA was excellent; while ovarian cancer cell DNA polymerase forms A–C pairs at a substantially higher frequency than its non-malignant cell counter-part. This suggested that the specificity of the substrate binding site within the DNA polymerases derived from non-malignant ovarian and ovarian cancer cells might be fundamentally distinct from one another. To examine this possibility we evaluated the kinetics of misincorporating dATP into nascent DNA as a function of dATP concentration for the DNA synthesome fraction derived from both of the NOSE cell lines and the three ovarian cancer cell lines.

Evaluation of the kinetics of nucleotide misincorporation by ovarian cancer cells

Using the approach described by Creighton and Goodman [28] for determining the fidelity of DNA synthesis mediated by purified DNA polymerase, our measurement of the frequency of misincorporation of dATP into nascent DNA by the synthesome fraction from the various ovarian cancer and non-malignant ovarian surface epithelial cell lines enabled us to examine the kinetics of dATP misincorporation using essentially Michaelis–Menten kinetics. By treating the frequency of misincorporation as a rate and examining this frequency of misincorporation as a function of the concentration of the nucleotide being misincorporated, we prepared a plot of (1/frequency of misincorporation) vs. 1/dATP concentration, and determined a V_{max}, and K_m for the misincorporation process for the DNA synthesome fraction in the two NOSE cell lines, and each of the three ovarian cancer cell lines [Fig. 2D]. Table 2 summarizes the results of our kinetic analysis examining the formation of a non-complementary A–C base pair by the DNA polymerases in the synthesome fraction from the non-malignant ovarian and ovarian cancer cell lines. The V_{max} for the reaction synthesizing a full-length nascent DNA containing an A–C base pair was approximately 4.2 ± 0.37 μmol/min for the synthesome fraction from both of the NOSE cell lines. Similarly the K_m for the reaction has an average value of 92.7 ± 12.7 μM. Our results indicate that the V_{max} for the DNA synthetic reaction mediated by the DNA synthesome fraction from the ovarian cancer cell lines was 127.4 μmol/min for the BG-1 cells, 120.4 μmol/min for the OVCAR-432 cells and 133.8 μmol/min for the Hey C2 cells. The K_m, for the DNA synthetic reaction mediated by the DNA synthesome fraction from the ovarian cancer cell lines was 294.1 μmol for the BG-1 cells, 409.3 μM for the OVCAR-432 cells and 407.0 μM for the Hey C2 cells. Our study shows that the average V_{max} (127.2 ± 6.7 μmol/min) for the synthetic reaction carried out by all of the ovarian cancer cell lines was approximately 30.7 fold higher than the average V_{max} (4.15 ± 0.37 μmol/min) for that of the NOSE cells ($P = 0.0001$). The average K_m (370.1 ± 65.7 μM) for the DNA synthetic reaction mediated by the DNA synthesome fraction from the ovarian cancer cell lines was approximately 3.99 fold higher than the average K_m (92.7 ± 12.7 μM) for that from the NOSE cells ($P = 0.0113$). According to Creighton and Goodman [28], the efficiency of forming a non-complementary base pair during elongation of a primed DNA template by the DNA synthesome fraction can be determined as the quotient of V_{max} / K_m. Our analysis of the efficiency for misincorporating dATP during the DNA synthetic reaction mediated by the non-malignant ovarian surface epithelial cells and the ovarian cancer cell lines indicated that the BG-1 cells were almost 4.3 times as likely to misincorporate an A across from a C nucleotide than its non-malignant cell counter-part; while the OVCAR-432 and Hey C2 cells were almost 6.5–7.2 times more likely to incorporate a non-complementary nucleotide base than the NOSE cells ($P = 0.0231$). These data suggest that the nucleotide

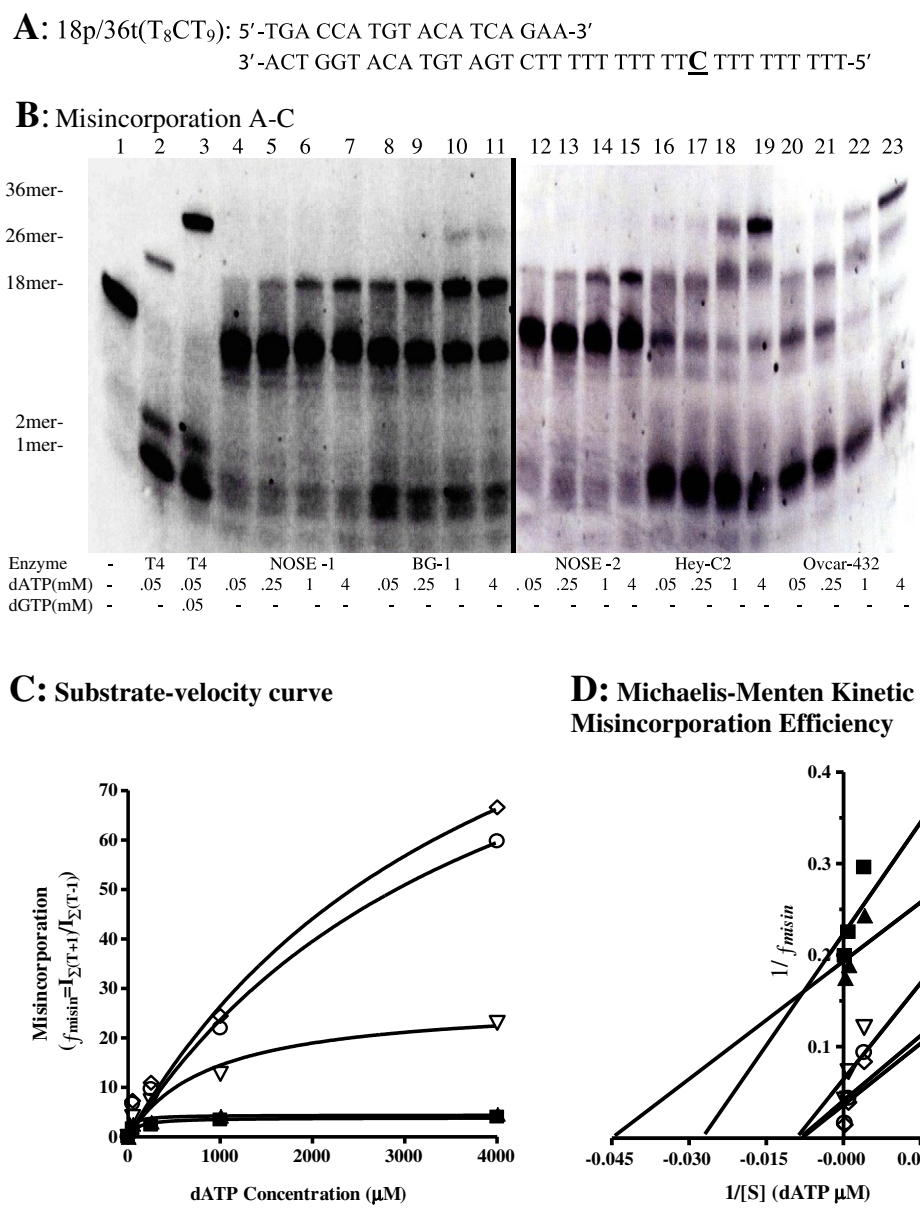

Fig. 2. Kinetic analysis of error-prone DNA synthesis mediated by the ovarian cancer cell DNA replication apparatus. Misincorporation of dATP into nascent DNA across from a template dCMP. (A) The nucleotide sequence of the 18 nt primer and 36 nt single stranded DNA template used in the primer extension assay. (B) PAGE analysis of the DNA primer extension reaction products formed by the DNA synthesome fraction from NOSE-1 (lanes 4–7); BG-1 (lanes 8–11); NOSE-2 (lanes 12–15); Hey C2 (lanes 16–19) and Ovcar-432 (lanes 20–23) cells. T4 phage DNA polymerase was substituted for the synthesome fraction in control reactions. Lane 1 contains only the primer template duplex and buffer. Lanes 2–3 contain primer template, T4 DNA polymerase, and one or both dNTP's required to fully extend the primed-template DNA duplex. The position of the labeled 18 nt primer, the 26 nt nascent DNA, and the 36 nt full length nascent DNA is shown to the left of the gel. The DNA synthesome fraction containing exonuclease activity cleaves primed DNA template to form 1–2 nt reaction products. The concentration of dATP and dCTP present in individual reactions is shown below the gel in panel B. (C–D) Michaelis–Menten kinetic analysis of the misincorporation frequency for each of the cell lines, as a function of the dATP concentration. The misincorporation frequency was determined by calculating the ratio of the fluorescent intensity of the 36 nt band relative to that of the 26 nt band. The panel on the left shows the frequency of misincorporation of dATP into the nascent DNA by each cell line. The panel on the right shows the results of the Michaelis–Menton kinetic analysis. The data from each cell line is denoted as: (■) NOSE-1; (▲) NOSE-2; (▽) BG-1; (○) Hey C2; (◊) OVCAR-432.

binding site within the ovarian cancer cell DNA polymerases has a lower affinity for the substrate nucleotide triphosphate than its non-malignant cell counterpart, and as a result can inappropriately pair dATP with a dCMP with a greater efficiency than non-malignant ovarian cell DNA polymerases.

Ovarian cancer cell DNA polymerase has a lower affinity nucleotide triphosphate binding site than its NOSE cell counter-part

To test the concept that the substrate nucleotide triphosphate binding site within the ovarian cancer cell DNA polymerases could be misincorporating non-complementary nucleotide triphosphates during DNA synthesis with a greater efficiency than their non-malignant cell counter-parts, we repeated the kinetic analysis described in Fig. 2, using the template shown in Fig. 3A. For this analysis, we used the 5′ fluorescently labeled 18 nt long single-stranded DNA primer and another 36 nt long single stranded DNA template containing a dGMP at position 27 in place of the dCMP within the 36 nt long DNA template used in the studies described in Fig. 2. The primer and template were annealed to one another over the first 18 nucleotides of the template as described in the Materials and methods section. The reaction mixture contained dGTP substituted for the dATP used in the previous experiment. The synthesome fraction from either the NOSE cells or the ovarian cancer cell lines were used in the assay.

Table 2
Kinetic analysis of nucleotide misincorporation by NOSE and ovarian cancer cells.

Cell line	Mis-incorporation of A–C			Mis-incorporation of G–G		
	V_{max}	K_m	E	V_{max}	K_m	E
	μM/min	μM	$\times 10^2$ min^{-1}	μM/min	μM	$\times 10^2$ min^{-1}
Non-malignant cells						
NOSE-1	3.88	101.7	3.81	4.41	83.7	5.26
NOSE-2	4.41	83.7	5.26	5.45	122.0	4.46
Average	4.15	92.7	4.54	4.93	102.9	4.86
SD	±0.37	±12.7	±1.03	±0.74	±27.1	±0.57
Malignant cells						
BG-1	127.4(30.7*)	294.1(3.2*)	19.3(4.3*)	33.7(6.8*)	276.2(2.7*)	19.1(3.9*)
Hey C2	133.8(32.2*)	407.0(4.4*)	32.8(7.2*)	36.6(7.4*)	272.1(2.6*)	13.4(2.8*)
OVCAR 432	120.4(29.0*)	409.3(4.4*)	29.4(6.5*)	22.5(4.6*)	281.7(2.7*)	18.0(3.7*)
Average	127.2(30.65*)	370.1(3.99*)	27.2(5.98*)	30.9(6.27*)	276.7(2.69*)	16.8(3.46*)
SD	±6.70	±65.7	±7.02	±7.45	±4.83	±3.02
P**	0.0001	0.0113	0.0231	0.0185	0.0013	0.0133

The values of V_{max}, K_m and the Efficiency ($E = V_{max} / K_m$) of nucleotide misincorporation are derived from the substrate–velocity curve, and are determined using GraphPad software (Prism). The symbol (*) shows the fold increase which is the ratio of the V_{max}, K_m and E value in the ovarian cancer cells and the average V_{max}, K_m and E in the normal ovarian surface epithelial (NOSE) cells. P** is a confidence interval assigned to the result of the *t* test used to compare the differences between the kinetic parameters for the ovarian cancer cells and the NOSE cells.

Bacteria phage T4 DNA polymerase was again used in the reaction as a control, and the reaction products formed by T4 DNA polymerase are shown in lanes 2–3 of the gel, Fig. 3B. The primer extension assay included either dGTP, as the sole nucleotide triphosphate, or both dGTP and dCTP. In the presence of only dGTP, the reaction extended the primer DNA to a length of 26 nt, while in the presence of both dGTP and dCTP, the primer was extended by T4 DNA polymerase to 36 nt. The concentration of dNTP's used in each of the reactions is indicated at the bottom of Fig. 3B, and quantification of the amount of labeled 26 nt and 36 nt long reaction product formed in the assay was performed as described [Materials and methods]. Full-length 36 nt reaction product can only form if a non-complementary G–G base pair is formed during the reaction. Lanes 1–3 serve as the reference lanes for identifying the relative position of the 18 nt primer, the 26 nt long initial reaction product, and the full-length 36 nt long reaction product within the polyacrylamide gel. The extent of misincorporation by the NOSE cell lines and the ovarian cancer cell lines is determined by quantifying the fluorescence intensity of the 26 nt and the 36 nt bands resolved by the gel. Each of the primer extension assays was performed at one of 4 concentrations of dGTP, in order to monitor the effect substrate concentration has on the amount of 26 nt and 36 nt product formed by the DNA synthesome preparations from each of the cell lines examined. As with the experiment shown in Fig. 2, the DNA synthesome fraction derived from the NOSE cell lines formed only a very small amount of full-length product (36 nt in length), with the bulk of the reaction product being 26 nt in length; while the DNA synthesome fraction from each of the ovarian cancer cell lines formed substantially more full-length (36 nt) product than the NOSE cells. Michaelis–Menten kinetic analysis of the frequency at which dGTP was misincorporated into full-length reaction product (Fig. 3D) resulted in a kinetic profile for misincorporation that closely resembled that shown for dATP misincorporation into newly synthesized DNA by the DNA synthesome from the NOSE and the ovarian cancer cell lines. Table 2 shows that both the K_m and V_{max} associated with the incorporation of dGTP into the nascent DNA product being synthesized by the DNA polymerases in the DNA synthesome preparation derived from the NOSE cells was essentially the same as that for the reaction described in Fig. 2. Similarly, the kinetic parameters associated with the synthesis of the full-length reaction product by the ovarian cancer cell DNA polymerases were 6.27 fold higher for the V_{max} ($P = 0.0185$), and 2.69 fold higher for the K_m ($P = 0.0013$), than that from the NOSE cells. The efficiency with which a G–G base pair forms in the full-length nascent DNA during the reaction with the ovarian cancer cell DNA polymerases was also 3.46 fold higher than that of the DNA polymerases from the NOSE cells ($P = 0.0133$). These data are consistent with our earlier observation that the nucleotide triphosphate binding site of the ovarian cancer cell DNA polymerases had a lower affinity for the nucleotide substrate than its NOSE cell counterpart. Also the ovarian cancer cell DNA polymerases misincorporated nucleotide triphosphates more efficiently than the NOSE cell DNA polymerases; suggesting that the ovarian cancer cell DNA polymerases are more error-prone than their non-malignant cell counter-parts.

Our observations summarized in Fig. 4 compare the efficiency with which the DNA polymerases from both the NOSE cells and the ovarian cancer cells create specific types of nucleotide mismatches. Both the NOSE-1 and NOSE-2 cell DNA polymerases exhibit a relatively high degree of fidelity during extension of a primed DNA template as compared to their cancer cell counterparts, and consequently exhibit a low efficiency for forming inappropriate purine–pyrimidine or purine–purine base pairs. In contrast, the DNA polymerases isolated from all of the ovarian cancer cell lines examined exhibit between 2.8 and 7.2 fold more nucleotide mismatches (G–G and A–C) during extension of a primed DNA template than their non-malignant cell counterparts; regardless of whether they were synthesizing a purine–purine or a purine–pyrimidine base pair. With regard to the efficiency of misincorporating nucleotides, the DNA polymerases from the ovarian cancer cells were more likely to create purine–pyrimidine nucleotide mismatches (4.3–7.2 fold higher) than the NOSE cell polymerase, while being only slightly more efficient (2.8–3.9 fold) at incorrectly pairing a purine nucleotide with a purine nucleotide.

Discussion

One of the hallmarks of human cancer is somatic mutations [29]. There are also numerous reports describing a wide variety of chromosomal alterations in cancer cells [30–32]. Herein we report on the relative fidelity of DNA synthesis in ovarian cancer, comparing tumor cell lines to normal ovarian surface epithelial cells (NOSE) grown in primary culture. Ovarian carcinomas are derived from ovarian surface epithelial cells and replication of damaged DNA in response to ovulation may be involved in the etiology of the cancer [33]. Our results reported here show that the DNA replication apparatus of ovarian cancers exhibits a propensity for carrying out an error-promoting DNA synthetic relative process to that of non-malignant ovarian cells.

The observed error-promoting DNA replication process of ovarian cancer cells appears to be inherent to these cells and not due to simply an increase in the rate of the synthetic activity of its DNA replication apparatus. The penchant of the ovarian cancer DNA replication apparatus for generating single nucleotide errors was demonstrated by the observation

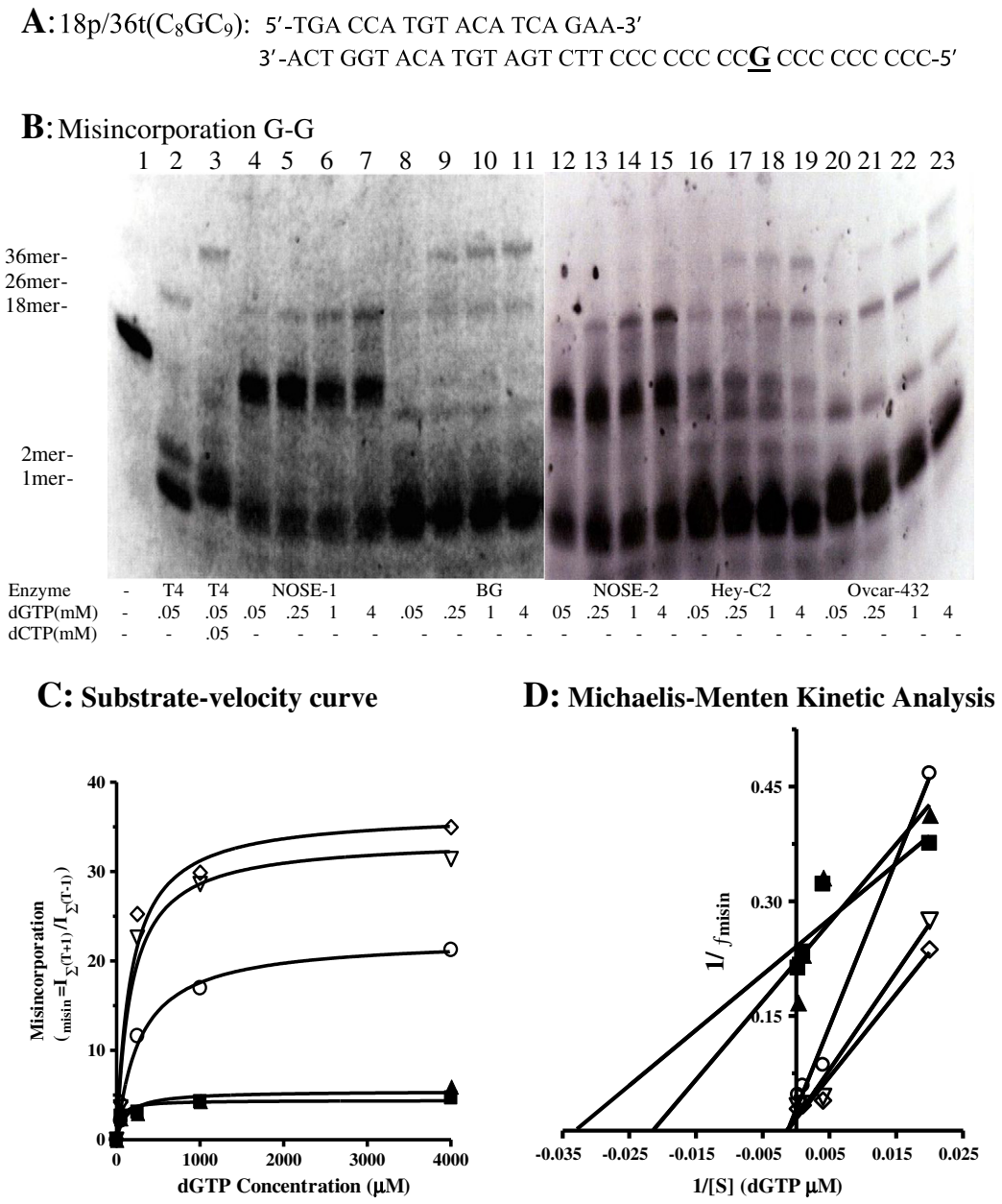

Fig. 3. Kinetic analysis of error-prone DNA synthesis mediated by the ovarian cancer cell DNA replication apparatus. Misincorporation of dGTP into nascent DNA across from a template dGMP. (A) The nucleotide sequence of the 18 nt primer and 36 nt single stranded DNA template used in the primer extension assay. (B) PAGE analysis of the DNA primer extension reaction products formed by the NE/S3 fraction from NOSE-1 (lanes 4–7); BG-1 (lanes 8–11); NOSE-2 (lanes 12–15); Hey C2 (lanes 16–19) and Ovcar-432 (lanes 20–23) cells. T4 phage DNA polymerase was substituted for the NE/S3 fraction in control reactions. Lane 1 contains only the primer template duplex and buffer. Lanes 2–3 contain primer template, T4 DNA polymerase, and one or both dNTP's required to fully extend the primed-template DNA duplex. The position of the labeled 18 nt primer, the 26 nt nascent DNA, and the 36 nt full length nascent DNA is shown to the left of the gel. The NE/S3 fraction containing exonuclease activity cleaves primed DNA template to form 1–2 nt reaction products. The concentration of dGTP and dCTP present in individual reactions is shown below the gel in panel B. (C–D) Michaelis–Menten kinetic analysis of the misincorporation frequency for each of the cell lines, as a function of the dATP concentration. The misincorporation frequency was determined by calculation the ratio of the fluorescent intensity of the 36 nt band relative to that of the 26 nt band. The panel on the left shows the frequency of misincorporation of dGTP into the nascent DNA by each cell line. The panel on the right shows the results of the Michaelis–Menton kinetic analysis. The data from each cell line is denoted as: (■) NOSE-1; (▲) NOSE-2; (▽) BG-1; (○) Hey C2; (◊) OVCAR-432.

that there is a difference in the efficiency with which the DNA polymerases associated with the DNA replication complex from both non-malignant ovarian surface epithelial cells and ovarian cancer cells create specific types of nucleotide mismatches during DNA synthesis. The "complexed" DNA polymerases derived from the non-malignant primary ovarian cells displayed a relatively high degree of fidelity during extension of a primed DNA template, as compared to the DNA polymerase from cancer cell lines; and consequently exhibit a low efficiency for forming inappropriate purine–pyrimidine or purine–purine base pairs. In contrast, the replication complex associated DNA polymerases isolated from all of the ovarian cancer cell lines examined exhibit more nucleotide mismatches during extension of a primed DNA template than their non-malignant ovarian cell counterparts, regardless of whether they were synthesizing a purine–purine or a purine–pyrimidine base pair.

DNA polymerase enzymes are fastidious in their selection of nucleotides during DNA synthesis, taking care that bases added to the growing DNA strand are correctly paired with their complements on the template strand (i.e., A's with T's, and C's with G's). However, these enzymes do make errors. Cells evolved sophisticated processes for fixing most, but not all, of these mistakes. Some of the mistakes are corrected during

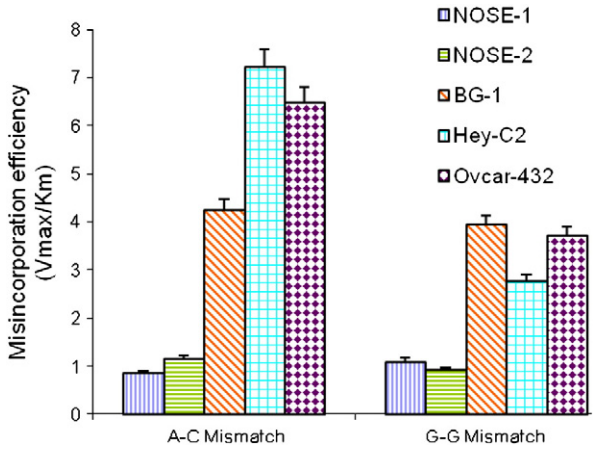

Fig. 4. DNA synthesome mediated nucleotide misincorporation efficiency in malignant and non-malignant ovarian cells. The efficiency of incorporating a non-complementary nucleotide into nascent DNA is determined using the kinetic analysis described in Fig. 2D.

DNA replication itself via the proofreading process, and some are corrected following replication in a process called mismatch repair. Regarding the observations described in this communication we may be able to distinguish between the error contributions generated in the ovarian cancer cells made by the replication process itself versus those potentially produced by a faulty mismatch repair process. The DNA synthesome is a purified multiprotein complex free of mismatch repair proteins. Therefore, the synthesome permits an observation of the replication process devoid the mismatch repair process. With this in mind, two possible mechanisms are suggested by the data presented in this paper for the potential contribution the ovarian cancer cell DNA replication apparatus makes to the accumulation of replication errors. The first is that the replicative polymerases in ovarian cancer cells are more error-prone with a decreased capacity to correctly select a nucleotide for incorporation into the growing DNA strand. Or second, the polymerase proof-reading process is faulty in ovarian cancer cells permitting replication errors to accumulate during DNA synthesis. Further studies are required to differentiate between these two possibilities. Nonethe-less, these explanations do not rule out the potential that in intact ovarian cancer cells there are additional avenues for the accumulation of increased base misincorporation that could entail insufficient detection and repair lesions occurring during DNA replication. Indeed there are published data to support a potential for DNA mismatch repair deficiency as contributory to the pathogenesis of some types of ovarian tumors [34–36].

The act of cell proliferation is what in essence makes us prone to developing cancer, and at the center of cell proliferation is the process of DNA replication. When a normal cell proliferates and in the process synthesizes new daughter DNA molecules, a low level of replication errors randomly occurs. However, genetic lesions that occur in proto-oncogenes, tumor suppressors, and/or mutator genes have a potential to transform the normal cell to a malignant one. Our data suggest that once transformation is established the ovarian cancer cell's DNA replication apparatus has an enhanced propensity for creating DNA synthetic errors, potentially leading to the accumulation of genetic lesions in genes that facilitate and drive ovarian cancer progression. An important question that needs to be pursued in the future would be to better understand how early in the ovarian cell transformation process does its DNA replication apparatus exhibit enhanced error propensity. This would better clarify the role of ovarian cancer cell DNA replication in disease progression.

Additional studies are needed to determine whether the recent work indicating that a significant number of serous carcinomas arise from the fallopian tube [37] is linked with the possibility that normal tubal cells display some propensity for error-prone DNA replication compared to NOSE. However, it should be noted that there are no current data to suggest that DNA replication occurs any differently, with any greater propensity to error in fallopian tubal epithelium, than any other epithelium in the body. None-the-less, this does require additional investigation.

A faithful DNA replication process is crucial to promote cell division and to limit cancer risk through the preservation of genome integrity [38]. There is a growing body of data that indicates that oncogene-induced alterations of the DNA replication program triggers replicative stress and replication-associated DNA damage; which favor the accumulation of genetic alterations in cancer cells [39–42]. It is therefore likely that key replication proteins and associated repair/recombination factors limit cancer development by preventing genomic instability during DNA replication. Previous work has demonstrated that the synthesome of both breast cancer and neuroblastoma cells has a significantly decreased DNA synthesis fidelity than that of the multiprotein complex of corresponding non-malignant cells [27,43] and these data complement the work described in this report. The data described in this manuscript represent the first direct experimental evidence indicating that the DNA synthetic machinery of ovarian cancer cells is itself mutagenic. It should also be noted that recent data on the DNA sequencing of a wide variety of human tumors have demonstrated that cancer cells contain thousands of mutations. These data support the concept that cancer cells express what has been termed a “mutator phenotype” [44]. The data described in this communication suggest that the acquisition of an error promoting DNA replication apparatus could be one mechanism used in cancer cells to support a mutator phenotype. One potential advantage an error promoting DNA replication apparatus could afford a cancer cell is an ability to adapt to a hostile host. The systematic analysis of the DNA replication process in ovarian cancer could uncover information on some of the molecular mechanisms that drive the accumulation of genetic damage, and probably contribute to the pathogenesis of the disease. This study suggests that a functionally altered DNA replication apparatus could be a previously unrecognized hallmark of ovarian cancer. Therefore, a structural and mechanistic characterization of the ovarian cancer DNA replication apparatus has the potential to provide insight into the process(es) whereby tumor cells can override some of the control signals, which regulate replication fidelity in non-malignant cells and contribute to the emergence of an error-promoting DNA replication process. It is our contention that by better understanding the pathogenesis of ovarian cancer, (i.e. by describing key molecular events in the development and maintenance of the disease), we may find improved strategies for the prevention, detection and treatment of ovarian carcinoma.

Conflict of interest statement

We agree to abide by *Gynecologic Oncology's* Confidentiality Policy. We agree to protect the integrity of the Journal by not using our positions as authors for *Gynecologic Oncology* in the commercial promotion of medical products or services. We agree to notify the Journal immediately if there has been any adverse action taken against our medical licenses in any jurisdiction. We agree to disclose any relevant financial relationships with commercial interests.

Acknowledgments

This work was supported in part by research awards from the National Institutes of Health/National Cancer Institute (R01 CA121289) to LHM and a research award from the Ovarian Cancer Research Foundation to RJH. In addition, research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under grant number P30CA033572. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors would also like to acknowledge the generous support of Moving Mountains for Midge. The authors would like to thank Angelica Ruiz and April Peigh for their assistance in the preparation of this manuscript and Brandy Snider for editorial assistance.

References

[1] Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol 2010;5:51–75.
[2] Masutani C, Kusumoto R, Yamada A, Dohmae N, Yokoi M, Yuasa M, et al. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. Nature 1999;399(6737):700–4.
[3] Kunkel TA. Considering the cancer consequences of altered DNA polymerase function. Cancer Cell 2003;3(2):105–10.
[4] Gallion HH, Powell DE, Morrow JK, Pieretti M, Case E, Turker MS, et al. Chromosome abnormalities in human epithelial ovarian malignancies. Gynecol Oncol 1990;38(3):473–7.
[5] Haapasalo H, Atkin NB, Collan Y, Pesonen E, Paljarvi L. Tumour ploidy, morphometry, histological grading and clinical features in ovarian carcinoma: mutual relations. Anal Cell Pathol 1991;3(5):261–71.
[6] Kulkarni AA, Loddo M, Leo E, Rashid M, Eward KL, Fanshawe TR, et al. DNA replication licensing factors and aurora kinases are linked to aneuploidy and clinical outcome in epithelial ovarian carcinoma. Clin Cancer Res 2007;13(20):6153–61.
[7] Kaern J, Trope CG, Kristensen GB, Abeler VM, Pettersen EO. DNA ploidy; the most important prognostic factor in patients with borderline tumors of the ovary. Int J Gynecol Cancer 1993;3(6):349–58.
[8] Ozalp S, Yalcin OT, Gulbas Z, Tanir HM, Minsin T. Effect of cellular DNA content on the prognosis of epithelial ovarian cancers. Gynecol Obstet Invest 2001;52(2):93–7.
[9] Pfisterer J, Kommoss F, Sauerbrei W, Rens H, duBois A, Jiechle-Schwarz M, et al. Cellular DNA content and survival in advanced ovarian carcinoma. Cancer 1994;74(9):2509–15.
[10] Reles AE, Gee C, Schellschmidt I, Schmider A, Unger M, Friedmann W, et al. Prognostic significance of DNA content and S-phase fraction in epithelial ovarian carcinomas analyzed by image cytometry. Gynecol Oncol 1998;71(1):3–13.
[11] Kallioniemi OP, Punnonen R, Mattila J, Lehtinen M, Koivula T, et al. Prognostic significance of DNA index, multiploidy, and S-phase fraction in ovarian cancer. Cancer 1988;61(2):334–9.
[12] Kallioniemi OP, Mattila J, Punnonen R, Koivula T. DNA ploidy level and cell cycle distribution in ovarian cancer: relation to histopathological features of the tumor. Int J Gynecol Pathol 1988;7(1):1–11.
[13] Loeb LA, Bielas JH, Beckman RA. Cancers exhibit a mutator phenotype: clinical implications. Cancer Res 2008;68(10):3551–7 [discussion 3557].
[14] Applegren N, Hickey RJ, Kleinschmidt AM, Zhou Q, Coll J, Wills P, et al. Further characterization of the human cell multiprotein DNA replication complex. J Cell Biochem 1995;59(1):91–107.
[15] Coll JM, Sekowski JW, Hickey RJ, Schnaper L, Yue W, Brodie AM, et al. The human breast cell DNA synthesom: its purification from tumor tissue and cell culture. Oncol Res 1996;8(10–11):435–47.
[16] Dai H, Liu J, Malkas LH, Hickey RJ. Characterization of RNA primers synthesized by the human breast cancer cell DNA synthesome. J Cell Biochem 2009;106(5):798–811.
[17] Jiang HY, Hickey RJ, Abdel-Aziz W, Tom TD, Wills PW, Liu J, et al. Human cell DNA replication is mediated by a discrete multiprotein complex. J Cell Biochem 2002;85(4):762–74.
[18] Lin S, Hickey R, Malkas L. The biochemical status of the DNA synthesome can distinguish between permanent and temporary cell growth arrest. Cell Growth Differ 1997;8(12):1359–69.
[19] Malkas LH, Hickey RJ, Li C, Pedersen N, Baril EF. A 21S enzyme complex from HeLa cells that functions in simian virus 40 DNA replication in vitro. Biochemistry 1990;29(27):6362–74.
[20] Ahluwalia A, Hurteau JA, Bigsby RM, Nephew KP. DNA methylation in ovarian cancer. II. Expression of DNA methyltransferases in ovarian cancer cell lines and normal ovarian epithelial cells. Gynecol Oncol 2001;82(2):299–304.
[21] Ahluwalia A, Yan P, Hurteau JA, Bigsby RM, Jung SH, Huang TH, et al. DNA methylation and ovarian cancer. I. Analysis of CpG island hypermethylation in human ovarian cancer using differential methylation hybridization. Gynecol Oncol 2001;82(2):261–8.
[22] Hurteau JA, Allison BM, Brutkiewicz SA, Goebl MG, Heilman DK, Bigsby RM, et al. Expression and subcellular localization of the cyclin-dependent kinase inhibitor p27(Kip1) in epithelial ovarian cancer. Gynecol Oncol 2001;83(2):292–8.
[23] Song J, Shih Ie M, Salani R, Chan DW, Zhang Z. Annexin XI is associated with cisplatin resistance and related to tumor recurrence in ovarian cancer patients. Clin Cancer Res 2007;13(22 Pt 1):6842–9.
[24] Bast Jr RC, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 1981;68(5):1331–7.
[25] Geisinger KR, Kute TE, Pettenati MJ, Welander CE, Dennard Y, Collins L, et al. Characterization of a human ovarian carcinoma cell line with estrogen and progesterone receptors. Cancer 1989;63(2):280–8.
[26] Dai H, Liu J, Malkas LH, Catalano J, Alagharu S, Hickey RJ, et al. Chromium reduces the in vitro activity and fidelity of DNA replication mediated by the human cell DNA synthesome. Toxicol Appl Pharmacol 2009;236(2):154–65.
[27] Sekowski JW, Malkas LH, Schnaper L, Bechtel PE, Long BJ, Hickey RJ, et al. Human breast cancer cells contain an error-prone DNA replication apparatus. Cancer Res 1998;58(15):3259–63.
[28] Creighton S, Goodman MF. Gel kinetic analysis of DNA polymerase fidelity in the presence of proofreading using bacteriophage T4 DNA polymerase. J Biol Chem 1995;270(9):4759–74.
[29] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646–74.
[30] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314(5797):268–74.
[31] Check Hayden E. Cancer complexity slows quest for cure. Nature 2008;455(7210):148.
[32] Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, et al. A census of human cancer genes. Nat Rev Cancer 2004;4(3):177–83.
[33] Murdoch WJ. Carcinogenic potential of ovulatory genotoxicity. Biol Reprod 2005;73(4):586–90.
[34] Suzuki, et al. DNA replication error is frequent in ovarian granulosa cell tumors. Cancer Genet Cytogenet 2000;122:55–8.
[35] Hirst, Brown. Detection of the replication error phenotype in ovarian cancer—PCR analysis of microsatellite instability. Methods Mol Biol 2001;39:375–82.
[36] Zhai QJ, Rosen DG, Lu K, Liu J. Loss of DNA mismatch repair protein hMSH6 in ovarian cancer is histotype-specific. Int J Clin Exp Pathol 2008;1:502–9.
[37] Auersperg N. The origin of ovarian cancers—hypotheses and controversies. Front Biosci (Schol Ed) 2013;5:709–19.
[38] Burhans WC, Carr AM, Wahl GM. DNA replication and cancer. In: DePamphilis M, editor. DNA replication and human disease. New York: Cold Springs Harbor; 2006.
[39] Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006;444(7119):633–7.
[40] Allen C, Ashley AK, Hromas R, Nickoloff JA. More forks on the road to replication stress recovery. J Mol Cell Biol 2011;3(1):4–12.
[41] Bartek J, Mistrik M, Bartkova J. Thresholds of replication stress signaling in cancer development and treatment. Nat Struct Mol Biol 2012;19(1):5–7.
[42] Zheng L, Dai H, Zhou M, Li X, Liu C, Guo Z, et al. Polyploid cells rewire DNA damage response networks to overcome replication stress-induced barriers for tumour progression. Nat Commun 2012;3:815.
[43] Sandoval JA, Grosfeld JL, Hickey RJ, Malkas LH. Structural analysis of the human neuroblastoma DNA replication complex: insights into faulty proliferation. J Pediatr Surg 2006;41:266–70.
[44] Loeb LA. Human cancers express mutator phenotypes: origin, consequences and targeting. Nat Rev Cancer 2011;6:450–7.