Translesion synthesis DNA polymerases $\eta$, $\iota$, and $\nu$ promote mutagenic replication through the anticancer nucleoside cytarabine

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Cytarabine (AraC) is the mainstay for the treatment of acute myeloid leukemia (AML). Although complete remission is observed in a large proportion of patients, relapse occurs in almost all the cases. The chemotherapeutic action of AraC derives from its ability to inhibit DNA synthesis by the replicative polymerases (Pols); the replicative Pols can insert AraCTP at the 3’-terminus of the nascent DNA strand, but they are blocked at extending synthesis from AraC. By extending synthesis from the 3’-terminal AraC and by replicating through AraC that becomes incorporated into DNA, translesion synthesis (TLS) DNA Pols could reduce the effectiveness of AraC in chemotherapy. Here we identify the TLS Pols required for replicating through the AraC templating residue and determine their error-proneness. We provide evidence that TLS makes a consequential contribution to the replication of AraC damaged DNA, that TLS through AraC is conducted by three different pathways dependent upon Pol$\eta$, Pol$\iota$, and Pol$\nu$, respectively, and that TLS by all these Pols incurs considerable mutagenesis. The prominent role of TLS in promoting proficient and mutagenic replication through AraC suggests that TLS inhibition in AML patients would increase the effectiveness of AraC chemotherapy; and by reducing mutation formation, TLS inhibition may dampen the emergence of drug resistant tumors and thereby the high incidence of relapse in AraC treated patients.

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells. In AML, myeloblast, an immature precursor of white blood cells in normal hematopoiesis, undergoes genetic changes that prevents its normal differentiation. The undifferentiated immature clone of myeloblasts continues to proliferate, jeopardizing the production of normal white blood cells. The replacement of normal blood cells with leukemia cells in bone marrow causes a large reduction not only in white blood cells but also in platelets...
and red blood cells. As a result, AML patients suffer from an increased risk of infection, anemia, and bleeding (1). The incidence of AML increases with age, and it accounts for about 90% of acute leukemias in adults. Cytarabine (1-β-D-arabinofuranosyl cytosine) (AraC) is a drug of choice in AML treatment (2-4). Although complete remission is observed in 50%-75% of cases, relapse occurs in almost all of these cases (5,6), requiring further treatment that may include post-remission chemotherapy, stem cells transplantation, or immunotherapy.

AraC differs from 2'-deoxycytidine by the presence of an additional hydroxyl group at the C2' position of the 2'-deoxyribose, and AraC differs from cytidine in that the 2' OH of the arabinose sugar points in a direction opposite to that of the 2' OH of the ribose sugar in ribonucleotides (Fig. 1A). The chemotherapeutic action of AraC derives from its ability to inhibit DNA replication. Inside the cell, AraC is converted to a triphosphate (7), and AraCTP competes with dCTP for incorporation into DNA. The replicative polymerases (Pols) can insert AraC into DNA, but they are inhibited at extending from AraC at the 3' terminus (8-10). In the next replication cycle, the presence of AraC in the template strand will be further inhibitory to DNA replication. However, human cells harbor a number of translesion synthesis (TLS) DNA Pols that can, in principle, surmount the chemotherapeutic action of AraC by both extending DNA synthesis from AraC terminated 3' ends and by replicating through AraC in the template strand. As such, TLS Pols may play a pre-eminent role in reducing the chemotherapeutic impact of AraC, but there exists little information on how the TLS Pols enhance the replication potential of AraC-treated cells.

Biochemical and structural studies have indicated that different TLS Pols are adapted for replicating through different types of DNA lesions and depending upon the DNA lesion, a particular TLS Pol may carry out only the insertion or the extension step of TLS, or it could perform both the steps of TLS (11). For example, among the Y-family Pols, Polη has the unique ability to accommodate two template residues in its active site and thus it can efficiently replicate through UV induced cyclobutane pyrimidine dimers (12-15). The ability of Polη to push the template purine A or G residue into a syn conformation and to form an Hoogsteen base pair with the incoming T or C, respectively, enables it to insert nucleotides (nts) opposite DNA lesions that impair Watson-Crick (W-C) base pairing (16-18). Rev1 pushes the templating G residue into a solvent filled cavity and an Arg residue in Rev1 forms hydrogen bonds with the incoming T or C, respectively, enables it to insert a C opposite N2-dG adducts which protrude into the DNA minor groove (21). Polκ is highly adapted for extending from such minor groove DNA lesions (22), whereas Polζ, a member of the B-family of Pols, can extend synthesis from the nt opposite from a large variety of DNA lesions (11,17,23-25).

Although we now have considerable information on how TLS Pols can replicate through different kinds of DNA lesions generated from exogenous and endogenous sources, this information pertains largely to DNA lesions that disrupt the base moiety and not the sugar moiety. In the absence of genetic and cellular studies, there is no clear understanding of the relevance of TLS in AraC-treated cells, and the identity of TLS Pols involved in AraC bypass is also unknown. As such, the role of TLS Pols in reducing the chemotherapeutic potential of AraC in AML remains undetermined. The mutagenic potential of TLS through AraC is also unknown. There is no information available on which TLS Pols function in an error-free manner and which in a mutagenic fashion. Since error-prone replication by TLS Pols through the AraC lesion may contribute to the emergence of drug resistant tumors and to the relapse of cancers, knowledge of which TLS Pols function in an error-prone manner could suggest means for preventing the emergence of drug resistant tumors and for reducing refractory AML.
Here we identify the TLS Pols required for replicating through AraC in human cells. Our data indicate that TLS through AraC occurs via three different pathways dependent upon Polo, Poli, and Polv, respectively. TLS through AraC generates a considerable level of mutagenesis as ~10% of TLS products harbor mutations in which an A is inserted opposite AraC, and all these Pols contribute to mutagenic TLS. The rather high mutagenicity of AraC may contribute to the emergence of drug resistant tumors and to the relapse of cancers.

Results

Genetic control of TLS through AraC in human cells

AraC (Fig. 1A) was incorporated in the lacZ target sequence in the leading strand in the SV40-based duplex plasmid system in which bidirectional replication initiates from a replication origin (Fig. 1B). Since the lacZ sequence in the AraC-containing strand is in frame, both error free and error prone TLS mechanisms generate blue colonies. The other DNA strand lacking AraC contains a +1 frameshift; hence, the lacZ gene in this strand is non-functional. The two DNA strands are further distinguished by the Kan+ (kanamycin resistance) gene in the AraC-containing strand and the Kan− gene in the other strand (Fig. 1B). In this plasmid system, the number of blue colonies among the total colonies that grow on LB + Kan plates gives a very reliable estimate of TLS frequency (26).

To identify the TLS Pols involved in replicating through AraC, we examined the effects of siRNA depletions on TLS frequency. In normal human fibroblasts (HFs) treated with control (NC) siRNA, TLS occurred with a frequency of ~47%, and depletion of Polk or the Rev3 catalytic subunit of Polv had no effect on TLS frequency (Table 1). Depletion of Polo or Poli, however, reduced TLS frequency to ~33%, and depletion of Polv conferred a reduction to ~27% (Table 1). To determine whether Pols η, i, and v carry out TLS independently or whether any two of these Pols function together in one TLS pathway, in which one Pol would insert a nt opposite AraC and the other Pol would extend synthesis from the inserted nt, we examined the effects of co-depletion of any two of these Pols on TLS frequency. Co-depletion of Polo and Poli reduced TLS frequency to ~23% and co-depletion of Polo with Polv reduced TLS frequency to ~19% (Table 1). The observation that co-depletion of Polo with Poli or with Polv conferred a greater reduction in TLS frequency than that seen upon their individual depletion suggested that Polo functions independently of Poli or Polv. The result that the co-depletion of Poli and Polv reduced TLS frequency to ~14% indicated that Poli and Polv function independently of one another (Table 1). Altogether, these results indicated that TLS through AraC is mediated via three independent pathways dependent upon Polo, Poli, and Polv, respectively.

To verify this conclusion, we examined the effects of depletion of Poli or Polv in XPV cells defective in Polo. In XPV cells, TLS through AraC occurs at a frequency of ~37% and it is reduced to ~25% in Poli depleted cells and to ~21% in Polv depleted cells (Table 2). This reduction in TLS frequency confirms the inference that Poli and Polv act independently of Polo. The observation that co-depletion of Poli and Polv in XPV cells reduced TLS frequency to ~7% (Table 2) adds further support to the conclusion that Pols η, i, and v function independently of one another in mediating TLS through AraC.

Mutagenicity of TLS opposite AraC

In HFs treated with control siRNA, TLS through AraC incurs significantly elevated mutagenicity, as ~9% of TLS products harbor a mutational change in which an A is incorporated opposite AraC (Table 3). Depletion of Polo or Poli reduced the frequency of mutagenic TLS to ~7.5%, and depletion of Polv increased the frequency of mutagenic TLS to ~14%. Similar to that in WT HFs treated with control siRNA, in HFs
depleted for Polh, Poli, or Polv, mutagenic TLS occurred via an A incorporation (Table 3). Even though all these Pols conduct error-prone TLS opposite AraC, the greater increase in mutation frequency in Polv-depleted cells than in Polh or Poli depleted cells suggests that Polv promotes a less error-prone mode of TLS opposite AraC than Polh or Poli.

Replication through AraC by purified TLS Pols

Our genetic evidence in human cells that TLS through AraC is mediated by Polh, Poli, and Polv dependent pathways and that all these Pols contribute to mutagenic TLS by inserting an A opposite AraC suggested that in biochemical assays these Pols will insert predominantly a G and less frequently an A opposite AraC. To assess this, we examined their ability for replicating through AraC in the presence of dATP, dTTP, dGTP, or dCTP, or all 4 dNTPs (Fig. 2). Opposite AraC, Polh inserts a G but it also inserts an A, T, or C; T or C, however, are inserted less well than an A (Fig. 2A). Opposite undamaged C, Polh exhibits high error-proneness, misincorporating all the nucleotides. In the presence of all 4 dNTPs, Polh replicates through AraC proficiently (Fig. 2A). In contrast to Polh, Poli inserts only a G opposite AraC and it is inhibited at extending synthesis any further (Fig. 2A). Polv also inserts only a G opposite AraC, and in the presence of all 4 dNTPs, it inserts a G and then extends synthesis (Fig. 2B).

TLS Pols promote survival of AraC treated cells

To determine the contribution of Polh, Poli, and Polv to survival in AraC treated cells, we incubated wild type HFs depleted for TLS Pols in media containing 30 µM AraC for 48h. Compared to cells treated with NC siRNA, survival was reduced to ~75% in cells depleted for Polh, Poli, or Polv, and co-depletion of Polh with Poli or Polv or co-depletion of Poli with Polv reduced survival to ~55% (Fig. 3A). In AraC treated XPV HFs, depletion of Poli or Polv reduced survival and co-depletion of Poli with Polv caused a further reduction in survival (Fig. 3B). Overall, all three Pols contribute about equally to the survival of AraC treated cells.

Discussion

Genetic pathways for replicating through AraC in human cells

From siRNA depletion of TLS Pols in normal HFs we inferred the involvement of Pols η, ι, and v in TLS through AraC, and based on co-depletion analyses in HFs and XPV cells, we concluded that these Pols act independently in replicating through AraC (Fig. 4). The proficient ability of purified Polh to insert a nt opposite AraC and to extend synthesis from the inserted nt suggests that Polh alone could conduct TLS through AraC. By contrast to Polh, Poli inserts a nt opposite AraC but it is very inefficient in extending synthesis from the inserted nt, suggesting that the extension step is performed by an as yet unidentified Pol. The proficient ability of Polv for inserting a nt opposite AraC and for extending synthesis suggests that Polv alone could replicate through AraC in human cells (Fig. 4).

High error proneness of TLS opposite AraC

In normal human cells, Pols η, ι, and v carry out highly error prone TLS opposite AraC. While the high error proneness of Polh for TLS in human cells conforms with the error prone synthesis by purified Polh opposite AraC, the error proneness of TLS by Pols ι and v in human cells is incongruent with the error free TLS performed by these purified Pols. The rather high error prone TLS by Pols ι and v in human cells and not with purified Pols stands in sharp contrast to the greatly reduced mutagenicity of TLS opposite other DNA lesions in human cells compared to the high error-proneness of TLS by purified Pols. Thus, e.g., TLS in human cells opposite UV induced cyclobutane pyrimidine dimers and (6-4) photoproducts, or opposite other DNA lesions such as...
thymine glycol, N3-methyladenine, N1-methyladenine, γ-hydroxy-1-N²-propano-2'-deoxyguanosine, and 1-N²-ethenodeoxyadenosine (26-34) occurs with a much higher fidelity than indicated from the fidelity of purified Pols. The much higher fidelity of TLS Pols in human cells than that indicated from in vitro biochemical analyses can be rationalized by assuming that TLS Pols in human cells are components of multi-protein ensembles and the fidelity of TLS Pols in these ensembles is actively modulated by protein-protein interactions and post-transcriptional modifications.

Contrawise, the acquisition of reduced fidelity by PolI and PolV in TLS opposite AraC in human cells would suggest that protein-protein interactions and post-transcriptional modifications contribute to reducing the fidelity of TLS Pols opposite AraC rather than to its enhancement. The striking divergence in the fidelity of TLS Pols opposite AraC vs. opposite other DNA lesions in human cells may accrue from the fact that all the other DNA lesions for which we have analyzed the genetic control and fidelity of TLS thus far, are generated from normal cellular reactions or from prevalent environmental sources; consequently, strong selection pressure for maintaining cellular homeostasis would have led to the acquisition of predominantly error-free TLS mechanisms. Since AraC is not a by-product of cellular reactions or generated from persistent environmental exposure, TLS mechanisms opposite this chemotherapeutic drug would have been under no selection pressure to adapt to a more error free mode; instead, the mechanisms which have evolved to adapt TLS Pols to act in a more error-free manner opposite the various more prevalent DNA lesions could have caused TLS Pols to operate opposite AraC in a more error-prone manner in human cells than in the purified Pol.

Role of TLS in countering the chemotherapeutic potential of AraC

TLS Pols will impact the chemotherapeutic potential of AraC first by extending synthesis from AraCMP at the 3'-terminus of the nascent DNA strand and second by replicating through AraC incorporated in the template strand. Biochemical studies have indicated a role of PolI in extending synthesis from AraC at the 3'-terminus and structural studies have shown that PolI can accommodate AraC via specific hydrogen bonding and stacking interactions (35). Our results that TLS Pols η, λ, and ν can promote replication through AraC and that their inactivation reduces survival of AraC treated cells suggest that TLS through AraC would contribute to reducing the effectiveness of AraC chemotherapy. In addition, our evidence that TLS through AraC generates a considerably high level of mutations explains the high mutagenicity conferred by AraC treatment (36-40), and suggests that TLS induced mutagenicity would contribute to the emergence of drug resistant tumors and to the relapse of cancers.

Experimental procedures

Construction of plasmid vectors containing an AraC and TLS assays

The 16-mer oligonucleotides containing an AraC were purchased from Trilink Biotechnologies, and the in-frame target sequences of the lacZ' gene in the resulting vector is shown in Fig. 1B. The wild type kanamycin gene was placed on the DNA strand containing AraC, and in this DNA strand lacZ' is in-frame and functional for β-galactosidase (β-gal). The opposite DNA strand harbors an SpeI restriction site containing a +1 frameshift which makes it non-functional for β-gal. Details of TLS assays have been published before (26,32).

Translesion synthesis assays in human cells and mutational analyses of TLS products

Wild type (GM637) or XPV (XP30RO) HFs were grown in DMEM media (GeneDepot) containing high D-glucose (4500 mg/L), Phenol red (15 mg/L), and sodium pyruvate (110 mg/L) with 10% FBS.
(GeneDepot) and plated in 6-well plates at 70% confluence (approximately, 3 x 10^5 cells per well). Cells were transfected with 100 pmole siRNAs with Lipofectamine 2000 (Invitrogen). For the simultaneous siRNA knockdown of two genes, 100 pmole siRNAs for each gene were mixed and transfected. After 48h incubation, the heteroduplex target vector DNA (1 μg) and 50 pmole of siRNA (second transfection) were cotransfected with Lipofectamine 2000 (Invitrogen). After 30 h incubation, plasmid DNA was rescued from the cells by the alkaline lysis method and digested with DpnI to remove unreplicated plasmid DNA. The plasmid DNA was then transformed into E. coli XL1-Blue super competent cells (Stratagene). Transformed bacterial cells were diluted in 1mL SOC medium and plated on LB/kan (25 μg/mL kanamycin) (Sigma). TLS analyses and mutational analyses of TLS products were carried out as described previously (26).

siRNA sequences used for knockdowns of human TLS Pols

The siRNA sequences used for knockdowns of Polη, Polâ, and Rev3 have been published previously (26). The siRNA sequence used for Polv depletion is 5'-CCCAAUCAGAUUACUACATT-3'.

AraC survival assay

Wild type (GM637) or XPV (XP30RO) HFs were transfected with siRNA and 48h after siRNA transfection, cells were incubated with 30 μM AraC (Sigma) in fresh growth media for 48h. AraC cytotoxicity was determined by MTS assay (Promega). Briefly, 100 μL of MTS assay solutions were added to each well containing cells and incubated for 30 min. Cell viability was determined by measuring OD at 490nM. Four independent experiments were carried out.

Protein expression and purification

Full length human Polη and Polâ were expressed as GST-tagged fusion proteins from plasmids pR30.186 and pPOL114, respectively, and purified from yeast cells as described (41-43). The GST tags were cleaved from each DNA Pol by treatment with prescision protease, leaving a 7 amino acid linker peptide at the N-terminus of each protein. To express human Polv lacking the proline rich C-terminal 39 residues (44) in yeast, a 2.6 kb E. coli codon optimized cDNA encoding amino acids 1-861 of the 900 amino acid protein was synthesized. The cDNA was amplified by PCR to add flanking 5' and 3' BamHI restriction endonuclease sites, and the fragment was cloned in frame with a Flag-Metal Affinity Tag (MAT)-SUMO* tag under control of a galactose inducible phosphoglycerate kinase (PGK) promoter in plasmid pPM1514, generating plasmid pBJ2086. SUMO* contains residues 1-98 of the yeast SMT3 encoded protein and harbors mutations R64E and R71E rendering it resistant to cellular sumo-proteases. Yeast strain YRP654 was transformed with plasmid pBJ2086 and cells were grown as described (41). Protein expression was induced by the addition of 2% galactose and cells were grown for 16 h. Polv(1-861) was affinity purified using anti-Flag agarose. Clarified protein extract was prepared from 10 g yeast cells disrupted by French press as described (41), except that breakage buffer additionally contained 0.1% Triton X100. The clarified protein extract was rocked with 0.1 ml M2 αFlag agarose (Sigma) for 3 h and subsequently washed with 20 volumes 1x GBB containing 500mM NaCl and 0.1% triton X100. The M2 αFlag agarose beads were equilibrated in 1x GBB containing 150 mM NaCl and 0.01% triton X100 and protein was eluted by the addition of 0.1 mg/ml flag peptide. Eluted protein was aliquoted and frozen at -70 C.

DNA polymerase assays

DNA substrates consisted of a radiolabeled oligonucleotide primer annealed to a 75 nt oligonucleotide DNA template by heating a mixture of primer/template at a 1:1.5 molar ratio to 95 °C and allowing it to cool to room temperature for several hours. The template
75-mer oligonucleotide contained the sequence 5′AGC AAG TCA CCA ATG TCT AAG AGT TCG TAT CAT GCC TAC ACT GGA GTA CCG GAG CAT CGT GAC TGG GAA AAC-3′ and it harbored an undamaged C or an AraC at the underlined position. For examining the incorporation of dATP, dTTP, dCTP, or dGTP nucleotides, or of all 4 dNTPs, a 44 mer primer 5′ GTT TTC CCA GTC ACG ACG ATG CTC CGG TAC TCC AGT GTA GGC AT-3′ was annealed to the above mentioned 75 mer templates.

The standard DNA polymerase reaction (5 μl) contained 25 mM Tris·HCl (pH 7.5), 5 mM MgCl₂, 1 mM dithiolthreitol, 100 μg/ml BSA, 10% glycerol, 10 nM DNA substrate, and 1 nM of Polη, Polι or Polv. For nucleotide incorporation assays with Polη or Polι, 25 μM dATP, dTTP, dCTP, or dGTP (Roche Biochemicals, Indianapolis) were used, and for examining synthesis through the undamaged C or AraC all 4 dNTPs (25 μM each) were used. For nucleotide incorporation assays with Polv, 10μM dATP, dTTP, dCTP, or dGTP were used, and for examining synthesis through the undamaged C or araC, all 4 dNTPs (10 μM each) were used. Reactions were carried out for 10 min at 37°C. Reaction products were resolved on a 12% polyacrylamide gel containing 8M urea and analyzed by a PhosphorImager.

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Conflicts of Interest - The authors declare that they have no conflicts of interest with the contents of this article.

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Table 1. Effects of siRNA knockdown of TLS Pols on the replicative bypass of the AraC lesion carried on the leading strand template in wild type HFs (GM637)

| siRNA       | No. Kan+ colonies | No. blue colonies among Kan+ | TLS (%) |
|-------------|--------------------|------------------------------|---------|
| NC          | 523                | 248                          | 47.4    |
| Polκ        | 316                | 154                          | 48.7    |
| Rev3        | 334                | 163                          | 48.8    |
| Polη        | 386                | 126                          | 32.6    |
| Polλ        | 402                | 134                          | 33.3    |
| Polν        | 418                | 114                          | 27.3    |
| Polη + Polλ | 378                | 86                           | 22.8    |
| Polη + Polν | 384                | 72                           | 18.8    |
| Polλ + Polν | 408                | 58                           | 14.2    |
Table 2. Effects of siRNA knockdown of TLS Pols on the replicative bypass of the AraC lesion carried on the leading strand template in XPV HFs (GM03617)

| siRNA      | No. Kan+ colonies | No. blue colonies among Kan+ | TLS (%) |
|------------|-------------------|------------------------------|---------|
| NC         | 433               | 162                          | 37.4    |
| Polα       | 350               | 86                           | 24.6    |
| Polβ       | 316               | 66                           | 20.9    |
| Polα + Polβ| 304               | 20                           | 6.6     |
Table 3. Effects of siRNA knockdown of TLS Pols on mutation frequency and nucleotide inserted opposite AraC carried on the leading strand template in wild type HFs (GM637)

| siRNA | No. of Kan⁺ blue colonies sequenced | A   | G   | C   | T   | Mutation frequency (%) |
|-------|-----------------------------------|-----|-----|-----|-----|------------------------|
| NC    | 448                               | 42  | 406 | 0   | 0   | 9.4                    |
| Polη  | 462                               | 34  | 428 | 0   | 0   | 7.4                    |
| Polα  | 355                               | 27  | 328 | 0   | 0   | 7.6                    |
| Polδv | 294                               | 40  | 254 | 0   | 0   | 13.6                   |
Figure legends

Figure 1.
TLS analyses opposite AraC.  A, Chemical structures of 2'-deoxycytidine, cytarabine, and cytidine.  B, The 16-mer target sequence containing an AraC is shown on top, and the lacZ sequence in the leading strand of the pBS vector containing the AraC nucleotide is shown below.  The AraC containing DNA strand is in-frame and it carries the Kan\(^+\) gene.  TLS through AraC generates Kan\(^+\) blue colonies

Figure 2.
Deoxynucleotide incorporation opposite C or AraC by TLS Pols.  A, Deoxynucleotide incorporation opposite undamaged C or AraC template by Pol\(\eta\) or Pol\(\iota\).  Each protein (1 nM) was incubated with DNA substrate (10 nM) and 25 \(\mu\)M each of a single deoxynucleotide dATP, dTTP, dGTP, or dCTP, or 25 \(\mu\)M each of all four dNTPs (N) for 10 min at 37 °C.  B, Deoxynucleotide incorporation by Pol\(\nu\) opposite undamaged C or AraC template.  Pol\(\nu\) (1 nM) was incubated with DNA substrate (10 nM) and with 10 \(\mu\)M each of a single nucleotide (A, T, G or C), or 10 \(\mu\)M each of all four dNTPs (N) for 10 min at 37°C.

Figure 3.
Effects of siRNA depletion of TLS Pols on survival of HFs treated with AraC.  A, GM637 (WT) HFs and B, Pol\(\eta\) defective XP30R0 HFs were depleted of TLS Pols, treated with 30 \(\mu\)M AraC, and cell survival determined by MTS assays.  Error bars indicate the standard deviation of results of 4 independent experiments.  Student’s two-tailed t-test P values: **, P<0.01; ***, p<0.001; ****, p<0.0001.

Figure 4.
TLS Pols promote error-prone replication through AraC in human cells.  TLS through AraC is mediated by the independent roles of Pol\(\eta\), Pol\(\iota\), or Pol\(\nu\).  Based on the proficiency of purified Pol\(\eta\) or Pol\(\nu\) for replicating through AraC, we suggest that each of these Pols carries out both the insertion and extension steps of TLS in human cells.  The inability of purified Pol\(\iota\) to extend synthesis from the nt it inserts opposite AraC suggests that an as yet unidentified TLS Pol carries out this role in human cells.
Figure 1

**A**

- 2'-Deoxycytidine, dC
- Cytarabine, AraC
- Cytidine, C

**B**

- 5'-GGA AGC AAT C GTACGG-3'

| Restriction Site | Sequence | Kan+ |
|------------------|----------|------|
| BamH I           | 5'-ATG ACC ATG ATT ACG GGA TCC GTG AAT GG TCC TGC AGG TCA CTG-3' | Kan+ |
| Spe I (+1 nt)    | 5'-TT GCT TCC TGC AGG TCA CTG-3' | Kan+ |
| Sbf I            | 3'-TAC TGG TAC TAA TGC CCT AGG GTA C T AA C GA AGG ACG TCC AGT GAC-5' | Kan+ |

Leading strand (pBS vector)
Figure 2
Figure 3
Error-proneness in human cells

Inserter Pol

Extender Pol

Figure 4
Translesion synthesis DNA polymerases η, ι, and ν promote mutagenic replication through the anticancer nucleoside cytarabine
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