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Mutagenic potential of hypoxanthine in live human cells

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ABSTRACT

Hypoxanthine (Hx) is a major DNA lesion generated by deamination of adenine during chronic inflammatory conditions, which is an underlying cause of various diseases including cancer of colon, liver, pancreas, bladder and stomach. There is evidence that deamination of DNA bases induces mutations, but no study has directly linked Hx accumulation to mutagenesis and strand-specific mutations yet in human cells.

Using a site-specific mutagenesis approach, we report the first direct evidence of mutation potential and pattern of Hx in live human cells. We investigated Hx-induced mutations in human nonmalignant HEK293 and cancer HCT116 cell lines and found that Hx is mutagenic in both HEK293 and HCT116 cell lines. There is a strand bias for Hx-mediated mutations in both the cell lines; the Hx in lagging strand is more mutagenic than in leading strand. There is also some difference in cell types regarding the strand bias for mutation types; HEK293 cells showed largely deletion (> 80%) mutations in both leading and lagging strand and the rest were insertions and A:T → G:C transition mutations in leading and lagging strands, respectively, whereas in HCT116 cells we observed 60% A:T → G:C transition mutations in the leading strand and 100% deletions in the lagging strand. Overall, Hx is a highly mutagenic lesion capable of generating A:T → G:C transitions and large deletions with a significant variation in leading and lagging strands in human cells. In recent meta-analysis study A → G (T → C) mutations were found to be a prominent signature in a variety of cancers, including a majority types that are induced by inflammation. The deletions are known to be a major cause of copy-number variations or CNVs, which is a major underlying cause of many human diseases including mental illness, developmental disorders and cancer. Thus, Hx, a major DNA lesion induced by different deamination mechanisms, has potential to initiate inflammation-driven carcinogenesis in addition to various human pathophysiological consequences.

1. Introduction

Hypoxanthine (Hx, the base component of 2′-deoxyinosine, dI) is a DNA lesion induced mainly by endogenous agents during chronic inflammation. Hx can be formed by different deamination mechanisms during inflammation. This includes simple DNA hydrolysis and reaction with nitrous anhydride (N_2O_3), a genotoxic derivative of nitric oxide [1–4]. However, nitric oxide-induced deamination of adenine to form Hx is not strongly supported [5]. Under inflammatory conditions in addition to deamination of adenine and formation of Hx (dI; Fig. 1A), guanine, cytosine and 5-methylcytosine are also deaminated to form xanthine, uracil and thymine, respectively [2,4]. Moreover, bases deaminate not only within the DNA molecule, but also as nucleoside triphosphates, precursors of DNA. Then a noncanonical nucleotide, such as dITP, is incorporated into the newly synthesized DNA, albeit less efficiently compared to dATP [6].

The level of Hx is detectable in animals (~5.7 lesions per 10^7 nucleotides), but it increases significantly during inflammation in infection-induced colitis and colon cancer models [7]. In general, DNA damage levels are known to be altered in cells by DNA repair pathways or through the replication process, which often convert DNA lesions to mutations [8–10].

Hx is known to be repaired efficiently by the base excision repair pathway. A monofunctional DNA glycosylase, N-methylpurine DNA glycosylase (MPG) recognizes Hx and efficiently excises the Hx base leaving a cytoxic abasic site (AP-site). AP endonuclease 1 (APE1) nicks the DNA at AP-site leaving a 5′-deoxyribose phosphate (dRP) moiety. A repair polymerase, DNA polymerase β fills in the nucleotide gap and removes the dRP. Finally, a DNA ligase seals the nick and completes Hx repair [10,11].

In several studies with *E. coli*, human cell line and transgenic mouse model, NO has been shown to have mutagenic activity under inflammatory conditions [12,13]. However, those studies did not examine Hx or other deaminated bases or direct relationship of deaminated bases with mutations. Moreover, no study has linked Hx accumulation to mutagenesis and strand-specific mutations in human

Abbreviations: Hx, hypoxanthine; 8-oxoG, 8-oxoguanine

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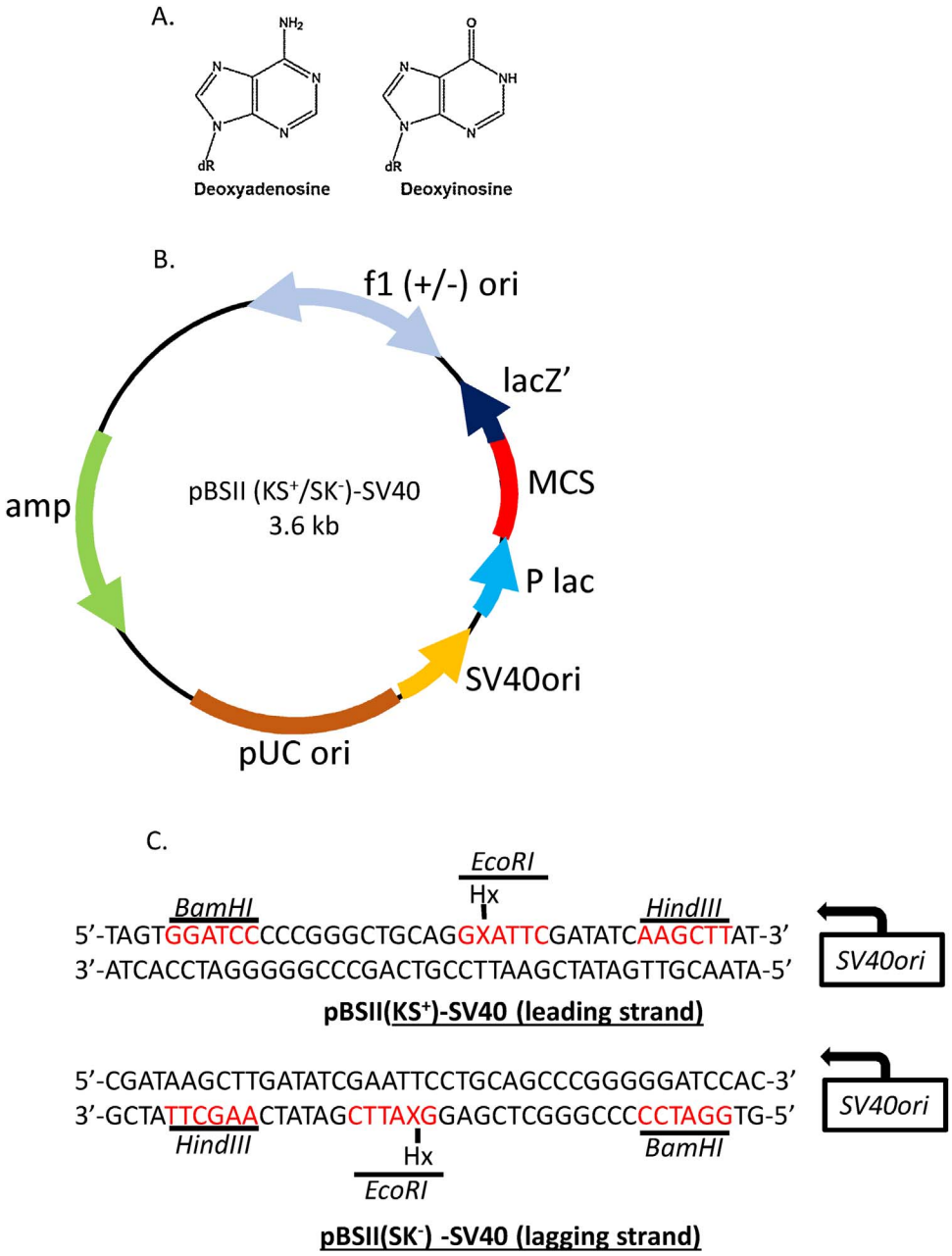

Fig. 1. Strategy for testing Hx-induced mutations on the leading or lagging strand DNA template of a duplex plasmid. A) Structures of deoxyadenosine (dA) and deoxyinosine (dI). B) The SV40-containing replicating shuttle vector used for the construction of plasmid DNA containing a site-specific Hx lesion. C) The target sequence containing Hx lesion at EcoRI site in the leading strand (pBSII(KS$^+$)-SV40) and the lagging strand (pBSII(KS$^-$)-SV40) are shown with respect to SV40 origin.

cells. We used a site-specific mutagenesis approach to investigate Hx-induced mutations in the replication context of both leading and lagging strands in human cells.

2. Materials and methods

2.1. Construct preparation and single-strand DNA (ssDNA) isolation

We constructed a replicating shuttle vector as previously described by Yoon et al. [14]. Essentially, the pBlusescript II (KS$^+$/SK$^-$)-SV40 (pBSII(KS$^+$/SK$^-$)-SV40) was prepared by cloning a DNA fragment (700 bp) containing SV40 replication origin sequence effectively in two opposite orientations with respect to the placement of Hx and 8-oxo-G at the EcoRI site within the multiple cloning site (MCS). The SV40 origin sequence was amplified from pSP189 using primers containing AflIII and SapI restriction sites and cloned in either pBSII (KS$^+$) or pBSII (SK$^-$) at AflIII and SapI sites. The phage suspension was prepared following a standard method [15]. Single-stranded pBSII(KS$^+$/SK$^-$)-SV40 DNA was isolated from the phage suspension using the Qiaprep Spin M13 kit and following manufacturer's protocol (Qiagen, Gaithersburg, MD).

Leading orientation was established when Hx was placed in the MCS (see below for method of Hx-containing DNA preparation) by primer extension on the KS$^+$ single stranded DNA (sense strand) so that the SV40ori is located on the 3′ side of Hx. During DNA synthesis, Hx is encountered by the polymerase while synthesizing the leading strand. Similarly, lagging orientation was established when Hx was placed in the MCS by primer extension on the SK$^-$ single stranded DNA (antisense strand) so that the SV40ori is on the 5′ side of Hx. Then during DNA synthesis the polymerase encounters Hx while synthesizing the lagging strand (Fig. 1B and C).

2.2. Hx- or 8-oxo-G- pBSII(KS$^+$/SK$^-$)-SV40 in vitro construct preparation

The modification (Hx or 8-oxo-G) was placed at the EcoRI site of the pBSII(KS$^+$/SK$^-$)-SV40 construct as described previously for 1, N^6