Heterozygous colon cancer-associated mutations of *SAMHD1* have functional significance

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Even small variations in dNTP concentrations decrease DNA replication fidelity, and this observation prompted us to analyze genomic cancer data for mutations in enzymes involved in dNTP metabolism. We found that sterile alpha motif and histidine-aspartate domain-containing protein 1 (SAMHD1), a deoxyribonucleoside triphosphate triphosphohydrolase that decreases dNTP pools, is frequently mutated in colon cancers, that these mutations negatively affect SAMHD1 activity, and that several *SAMHD1* mutations are found in tumors with defective mismatch repair. We show that minor changes in dNTP pools in combination with inactivated mismatch repair dramatically increase mutation rates. Determination of dNTP pools in mouse embryos revealed that inactivation of one *SAMHD1* allele is sufficient to elevate dNTP pools. These observations suggest that heterozygous cancer-associated *SAMHD1* mutations increase mutation rates in cancer cells.

dNTP pools | colon cancer | DNA replication fidelity

Recent advances in whole-genome sequencing have revealed that human cancers often contain thousands of subclonal mutations (1–3), lending support to the mutator phenotype hypothesis that postulates that an elevation in spontaneous mutation rate is an early step in cancer evolution (4, 5). The three major determinants of DNA replication fidelity that control the spontaneous mutation rate are nucleotide selectivity by DNA polymerases, proofreading by replicative DNA polymerases, and the mismatch repair (MMR) system (6). Failures in the two latter determinants have now been firmly associated with the development of cancer (7), but they cannot account for the increased spontaneous mutation rates in most cancers (5).

The first determinant, nucleotide selectivity by DNA polymerases, can be affected by changes in the absolute and relative concentrations of the four deoxyribonucleoside triphosphates (dNTPs). We have previously demonstrated that severely imbalanced dNTP pools strongly decrease DNA replication fidelity in *Saccharomyces cerevisiae* (8, 9) without affecting cell proliferation, as long as none of the dNTPs is limiting for DNA replication (10). An equimolar elevation in dNTP pools also decreases DNA replication fidelity, both in yeast and bacteria, presumably by suppressing the proofreading activity of replicative DNA polymerases and by stimulating lesion bypass by both replicative and translesion DNA polymerases (11–16). Recently, we showed in yeast that even a small elevation of the dNTP pool dramatically decreases the replication fidelity of exonuclease-deficient DNA polymerase ε (Pol ε) and DNA polymerase δ (Pol δ) harboring the cancer-associated R696W mutation (17–19). Based on these observations, we hypothesized that decreased nucleotide selectivity caused by changes in the absolute or relative concentrations of dNTPs could be one of the reasons for the increased mutation rates in cancers.

The absolute and relative concentrations of dNTPs are controlled by several dozen proteins (20), and mutations or a change in abundance in any of these could in principle result in a distortion of the dNTP pool. Ribonucleotide reductase (RNR), dCMP deaminase, dUTPase, dTMP synthase, dTMP kinase, and NDP kinases control dNTP biosynthesis. Purine and pyrimidine de novo synthesis pathways provide substrates for RNR, and multiple (deoxy)nucleoside kinases and 5′ nucleotidases control cellular and mitochondrial dNTP salvage. We analyzed the mutation status of the genes involved in dNTP metabolism in colon cancers using a public dataset from The Cancer Genome Atlas (TCGA) and identified *SAMHD1* (sterile alpha motif and histidine-aspartate domain-containing protein 1) as one of the frequently mutated genes.

SAMHD1 is a dual-function enzyme with both nuclease and deoxyribonucleoside triphosphate triphosphohydrolase (dNTPase) activities (21, 22). Germ-line mutations in *SAMHD1* have been associated with Aicardi–Goutieres syndrome, a congenital autoimmune disease (23), and more recently SAMHD1 was shown to be an HIV-1 restriction factor operating in nondividing blood cells (24, 25). Initially, the restriction function of SAMHD1 was attributed to its dNTPase activity, which was presumed to decrease the intracellular dNTP concentrations to levels incompatible with viral replication (26). Later, it was suggested that restriction of HIV-1 was primarily caused by the nuclease activity of SAMHD1 degrading viral RNA (27). However, more recently it was proposed that SAMHD1 lacks nuclease activity altogether and that the restriction of HIV-1 is caused by alternating ssRNA-binding and dNTPase activities (28). Franzolin et al. showed that SAMHD1 is expressed in a cell cycle-regulated manner and that loss of SAMHD1 has large effects on dNTP pool composition in vitro in both quiescent and cycling cells (29). *SAMHD1* has also been identified as a potential driver gene in chronic lymphatic leukemia, where it is recurrently mutated in early stages of tumor development (30–32). In solid tumors, lower

Significance

The three major DNA replication fidelity determinants are nucleotide selectivity, proofreading, and mismatch repair. Defects in the two latter determinants are now firmly associated with cancer. Nucleotide selectivity is affected by changes in the absolute or relative concentrations of dNTPs. Here, we show that hemizygous *SAMHD1*$^{+/-}$ mouse embryos have increased dNTP pools compared with wild-type controls and that heterozygous mutations that inactivate SAMHD1 are frequently found in colon cancers. We infer that such cancer cells have increased dNTP pools and, therefore, higher mutation rates. These observations suggest that changes in dNTP concentrations, which affect nucleotide selectivity, the first major determinant of DNA replication fidelity, are associated with cancer.

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protein and RNA expression of *SAMHD1* has been observed, presumably caused by promoter methylations (30, 33, 34). However, it is unknown whether *SAMHD1* somatic mutations found in cancers affect its dNTPase activity (35).

Here, we show that colon cancer-associated mutations in SAMHD1 either abolish its dNTPase activity or change its specificity, leading to unequal degradation of individual dNTPs. Importantly, even a hemizygous deletion of *SAMHD1* leads to an increase of dNTP pools in mouse embryos, which, similar to tumors, contain actively dividing cells. This result suggests that, although the *SAMHD1* mutations identified in cancers are heterozygous, they would still result in an alteration of dNTP pools. Interestingly, several of the identified *SAMHD1* mutations were present in MMR-deficient hypermutated cancers. Analysis of MMR-deficient yeast strains and human colorectal carcinoma cells demonstrated that even a small alteration of dNTP pools results in a multiplicative increase of mutation rates. Together, these findings suggest that mutations affecting the activity of SAMHD1 are likely to result in increased mutation rates in cancer.

Results

***SAMHD1* Is Frequently Mutated in Colon Cancer.** Mutations in *SAMHD1* have previously been associated with chronic lymphatic leukemia, in which *SAMHD1* has been suggested to be one of the driver genes, but its role in solid cancers is uncertain (30–32). The Catalogue of Somatic Mutations in Cancer (COSMIC) currently contains 104 *SAMHD1* mutations (36), and a quarter of these mutations were found in tumors of the large intestine (26 mutations in 24 samples). With the exception of one 31-nucleotide deletion, all *SAMHD1* mutations in tumors of the large intestine were single-nucleotide polymorphisms (Table S1). The majority of the mutations were predicted to be deleterious by using in silico analysis: 87% were predicted to be damaging by at least one of four computational tools (Grantham, SIFT, PolyPhen2, and CADD), 72% by two or more tools, and 50% by three tools (Table S1) (37–40). Four of the mutations (R145, D207, R366, and R451) are located at amino acid residues that have been reported to have functional significance in in vitro studies (27, 41, 42).

Eight of the 26 *SAMHD1* mutations (Table S1) were found in the publicly available colorectal cancer (CRC) TCGA dataset, hgsc.bcm.edu COAD.IlluminaGA DNASeq.Level 2.1.5.0, which was downloaded for further analysis. The dataset contained 114,594 mutations in 217 tumors; thus, 3.7% (8 of 217) of the tumors in this CRC dataset carried a coding mutation in SAMHD1. Approximately 20% of the 217 tumors were hypermutated (>12 mutations per 10^6 bases) (43), and the eight *SAMHD1* mutations (Table S1) were found in these hypermutated tumors. Analysis of the dataset using the Significantly Mutated Genes (SMG) test from the MuSIC suite (44) showed that—when taking into consideration mutation type and gene length—*SAMHD1* carried more mutations than expected by chance (P = 0.049, false discovery rate = 0.05, convolution test).

Of the total number of mutations falling within coding regions of all genes in the CRC dataset, 39% were silent, whereas none of the eight mutations in *SAMHD1* were silent. Among the 26 *SAMHD1* mutations in the current COSMIC database, only 2 (8%) were silent mutations. An increase in the ratio between nonsilent and silent mutations in a gene can indicate that functional mutations within that gene are beneficial for the tumor and are selected for during cancer development. Analysis of a panel of genes of known genetic importance in colon cancer (*APC*, *BRAF*, *KRAS*, *PIK3CA*, *SMAD4*, and *TP53*) (45, 46) also showed a lower frequency of silent mutations (3.4%) compared with the overall frequency of silent mutations (39%).

Collectively, these data indicate that *SAMHD1* mutations in colon cancer occur nonrandomly and affect functionally important residues, suggesting that deleterious mutations in this gene can be beneficial for the tumors.

CRC-Specific Mutations Negatively Affect SAMHD1 dNTPase Activity. To investigate the effect of the CRC-specific amino acid substitutions on SAMHD1 dNTPase activity, we chose four mutations (V133I, A338T, R366H, and D497Y) for recombinant protein purification and characterization (Fig. S1). V133I was selected because two tumors carried this mutation, but it was unclear from the in silico analysis to what extent V133I would affect the activity of SAMHD1. The other three mutations were predicted to be deleterious, and we wanted to confirm this prediction biochemically.

To measure the dNTPase activity, we quantified the deoxyribonucleoside production derived from the hydrolysis of dNTPs by the wild-type (WT) SAMHD1 protein and the four mutants over a time course of 60 min (Fig. S2). Compared with WT, all mutants had reduced specific dNTPase activity toward all four dNTPs (Fig. 1). Interestingly, whereas the D497Y mutation nearly abolished the dNTPase activity, other mutations had varying effects on SAMHD1 activity toward the individual dNTPs. For example, there was a ninefold difference between deoxyadenosine and deoxyguanosine production by the R366H variant of SAMHD1 (Fig. 1*D*). These data suggest that *SAMHD1* mutations might result in imbalanced dNTP pools.

Hemizygous *SAMHD1* Deletion Leads to Elevated dNTP Pools in Mouse Embryos. All eight identified CRC-associated SAMHD1 mutations from the TCGA dataset were heterozygous, and the corresponding methylation data from the TCGA database did not show any SAMHD1 promoter methylation that might indicate inactivation of the WT allele. Thus, although the four tested mutations—V133I, A338T, R366H, and D497Y—negatively affected SAMHD1 dNTPase activity in vitro, it was not clear whether these heterozygous mutations would have any effect on dNTP pools in vivo. To address this question, we compared dNTP pools in WT, SAMHD1$^{+/-}$ hemizygous, and SAMHD1$^{-/-}$ homozygous embryonic day 13.5 (E13.5) mouse embryos. We chose

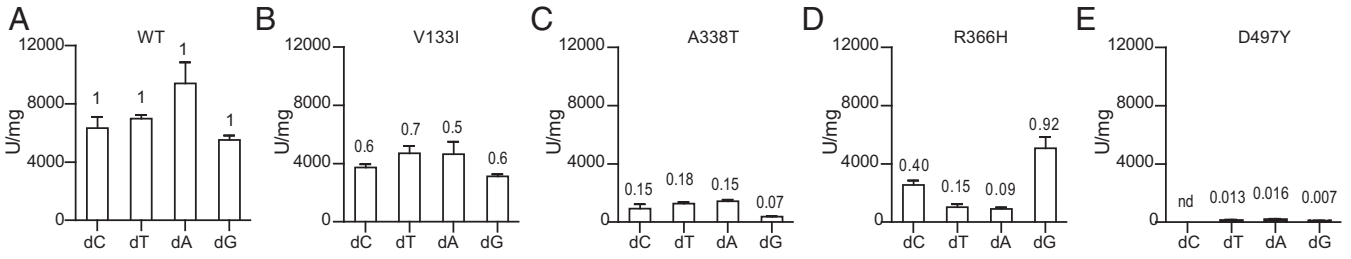

Fig. 1. In vitro dNTPase activity of SAMHD1 is altered by cancer-associated mutations. Purified SAMHD1 (*A*) and SAMHD1 mutant proteins V133I (*B*), A338T (*C*), R366H (*D*), and D497Y (*E*) were incubated with 1 mM dCTP, dTTP, dATP, or dGTP separately with 1 mM GTP as the activator, and the deoxynucleoside products (dC, dT, dA, and dG) were analyzed by HPLC. Numbers indicate detected deoxynucleoside products in mutants compared with WT SAMHD1. Error bars indicate SD. nd, not detectable.

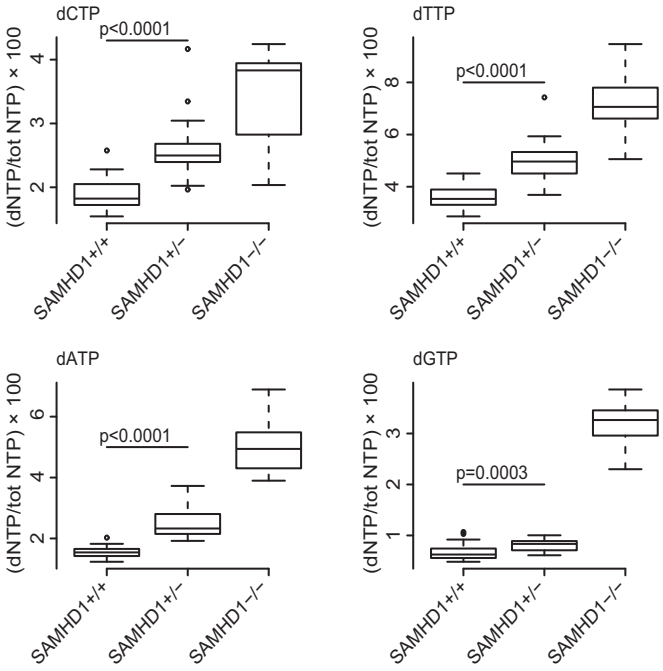

Fig. 2. dNTP levels in mouse embryos are affected by *SAMHD1* copy number. dNTP levels were measured in E13.5 mouse embryos that were WT (33 embryos), lacking one copy of *SAMHD1* (13 embryos), or lacking both copies of *SAMHD1* (18 embryos). Results are presented in a boxplot where the central box spans the first to the third quartile, the whiskers represent minimum and maximum values, and the segment inside the box is the median. Outliers are represented by circles. The significance value was calculated by using the Wilcoxon rank sum test.

whole embryos because they contain a large number of actively dividing cells, similar to actively proliferating solid tumors. As expected, dNTP pools were significantly increased in homozygous SAMHD1$^{-/-}$ embryos. Importantly, hemizygous SAMHD1$^{+/-}$ embryos also had elevated and imbalanced dNTP pools compared with WT, and dCTP and dTTP were increased by ∼40%, dATP by ∼60%, and dGTP by ∼20% (Fig. 2). These data strongly suggest that CRC-associated heterozygous SAMHD1 mutations that negatively affect its dNTPase activity in vitro should result in an elevation of dNTP pools in vivo.

Small Alteration of dNTP Pools Dramatically Increases Mutation Rates in Combination with MMR Deficiency. Further analysis of the eight tumors with mutated *SAMHD1* showed that six of them had defects in MMR (Table 1 and Fig. S3). To model the effect of minor dNTP pool increases on DNA replication fidelity in the absence of MMR, we used a *S. cerevisiae* strain containing a deletion of the *MSH2* gene and the *rnr1-Y285F* allele. Y285 is located in the allosteric specificity site of Rnr1, the large subunit of RNR. We have previously demonstrated that *rnr1-Y285F* alone leads to a slightly imbalanced elevation of dNTPs (3× dCTP, 3× dTTP, 1.8× dATP, and 1× dGTP) that in turn results in a ∼2.5-fold increase in mutation rate compared with WT (10), whereas *msh2*Δ alone increases the mutation rate ∼15-fold compared with WT. The *msh2*Δ *rnr1-Y285F* double mutant tested in this work had a ∼45-fold increased mutation rate compared with WT, demonstrating that minor changes in dNTP pools have a multiplicative effect in combination with the loss of MMR (Fig. 3 *A* and *B*).

In mammalian cells, severe dNTP pool imbalances are known to be mutagenic (47, 48), but to what extent minor dNTP pool changes increase mutation rates is not well known. To model the effect of minor dNTP pool changes on DNA replication fidelity in human cancer cells, we measured mutation rates at the *HPRT* locus of the HCT116 colorectal carcinoma cell line that was manipulated to have altered dNTP pools. This cell line lacks functional MMR due to a homozygous mutation of *MLH1* (49), and it is commonly used for the analysis of mutation rates under different conditions (50–52). To alter intracellular dNTP pools in this cell line, we supplemented the cell culture medium with deoxyribonucleosides, which are stepwise phosphorylated by deoxynucleoside (dN) kinases and deoxyribonucleotide kinases into dNTPs (20). Addition of one deoxyribonucleoside at high concentrations results in a dramatic increase in one or several dNTPs and a depletion of one or several other dNTPs because of the allosteric regulation of RNR. For example, addition of 1 mM thymidine results in a ∼25-fold increase of dTTP and a ∼10-fold decrease of the dCTP pool and leads to S-phase arrest (53). This effect of thymidine is commonly used for synchronization of cells by the so-called double thymidine block. Therefore, we sought to identify conditions in which the addition of several deoxyribonucleosides at lower concentrations would result in a minor change of dNTP pools without a concomitant S-phase arrest. We first tested thymidine at a concentration of 0.5 mM, which resulted in an 11-fold increase in dTTP and a 5-fold decrease in dCTP (Fig. S4*A*). Then, we tested thymidine at a concentration of 0.05 mM, which resulted in a 2.8-fold increase in dTTP and a 2-fold decrease in dCTP (Fig. S4*B*). We then tested the addition of deoxyadenosine alone and in combination with thymidine and found that, in the presence of 50 μM thymidine and 20 μM deoxyadenosine, dTTP and dATP increased approximately twofold and dCTP and dGTP levels decreased approximately twofold (Fig. S4*B* and Fig. 3*C*). At these concentrations of dN, flow cytometry analysis did not demonstrate any S-phase arrest (Fig. 3*D*). Therefore, we decided to proceed with measurements of mutation rates in the presence or absence of 50 μM thymidine and 20 μM deoxyadenosine, according to the schematic in Fig. S5. Mutation frequencies were invariably increased in the cell cultures grown in the presence of 50 μM thymidine and 20 μM deoxyadenosine (Fig. 3*E*), whereas the population-doubling (PD) time and cell-cycle progression were similar to the control cells grown in the absence of deoxyribonucleosides (Fig. S6). Mutation rates calculated by using mutation frequencies and PD times (Fig. 3*E*) were approximately threefold higher in the cells grown with exogenous deoxyribonucleosides (4.46×10^{-5} vs. 1.45×10^{-5}).

Discussion

Are dNTP pools altered in cancer cells compared with dNTP pools in normal cells? And, if so, do changes in dNTP pools contribute to the increased mutation rates and development of cancer? These are not easy questions to answer. dNTP pools are orders of magnitude lower than the corresponding NTP pools (54) and are, for that reason, difficult to measure. Publications reporting that cancer tissues have elevated dNTP pools compared with normal surrounding tissues are not informative because dNTPs are

Table 1. MMR status in the eight tumors from the TCGA dataset with *SAMHD1* mutation

Amino acid substitution	MMR heterozygous LOF	*MLH1* methylation
V133I_1	*MLH3* (fs)	*MLH1* (d)
V133I_2	*MLH1* (fs)	
A338T		
A338V	*MLH3* (fs)	*MLH1* (d)
R366H	*MLH1* (fs)	*MLH1* (s)
D497Y	*MLH3* (ns)	
A525T	*MLH1* (fs)	
K596fs		

Loss-of-function mutations (LOF) in *MLH1*, *MLH3*, *MSH2*, and *MSH6* and promoter methylation of *MLH1* were included in the analysis. d, both alleles methylated; fs, frameshift; ns, nonsense; s, one allele methylated.

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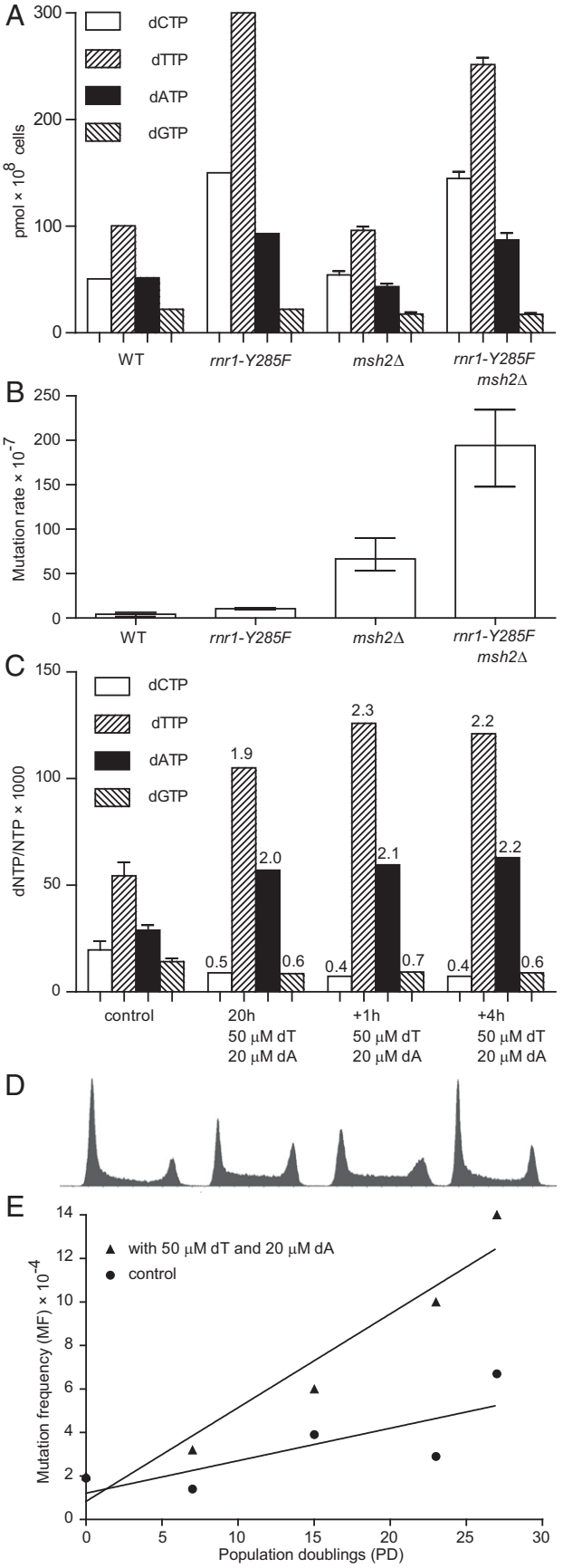

Fig. 3. Minor alterations of dNTP pools further elevate mutation rates in MMR-deficient cells. (*A* and *B*) Amount of each dNTP (*A*) and mutation rates (*B*) in the WT, *msh2*Δ, *rnr1*-Y285F, and *msh2*Δ *rnr1*-Y285F yeast strains. (*C*) Amount of each dNTP normalized to the total NTP pool in untreated HCT116 cells (control), HCT116 cells incubated in the presence of 50 μM thymidine and 20 μM deoxyadenosine for 20 h (20 h), and in HCT116 cells incubated in the presence of 50 μM thymidine and 20 μM deoxyadenosine for 20 h, after which an additional 50 μM thymidine and 20 μM were added. dNTP pools were measured after 1 h (+1 h) and after 4 h (+4 h). (*D*) Flow cytometry histograms of the HCT116 cells used for dNTP pool measurements in *C*. (*E*) Mutation frequencies and PDs of the HCT116 cells incubated in the presence or absence of 50 μM thymidine (dT) and 20 μM deoxyadenosine (dA).

produced primarily during S phase. Thus, dNTP pools are high in mitotic cells, such as cancer cells, and low in nondividing cells. Comparisons of dNTP pools in cancerous and normal immortalized cell lines in vitro also have caveats. First, the mitotic index, and thus the proportion of S-phase cells containing elevated dNTP pools, is often higher in cancerous cell lines. Second, the volume of cells in different cell lines might vary. An apparent increase in dNTP pools in cancerous cells will not result in a higher intracellular dNTP concentration if the volume of such cells is also increased compared with normal cells. Finally, dNTP pools in different cell lines in vitro are affected by the nucleosides or bases present in the culture medium (Fig. S4) (55). These dNTP precursors can be taken up with different efficiencies depending on the status of the salvage enzymes, such as thymidine kinase, deoxyguanosine kinase, and deoxyadenosine kinase, in cancerous vs. normal cells.

Here, we propose that some colon cancers have altered intracellular dNTP pools. We base our conclusion on the following observations: (*i*) These cancers have mutations in SAMHD1, an enzyme responsible for the degradation of dNTPs; (*ii*) the identified *SAMHD1* mutations negatively affect its dNTPase activity in vitro; and (*iii*) actively dividing hemizygous *SAMHD1*$^{+/-}$ mouse embryos have increased dNTP pools compared with congenic WT embryos of the same age. Previous dNTP pool measurements were performed in various types of WT and *SAMHD1*$^{-/-}$ homozygous cells, including E14.5 mouse embryonic fibroblasts (56, 57), but, to our knowledge, ours is the first study to measure dNTP pools in hemizygous *SAMHD1* mouse embryos. This observation is important because it demonstrates that cancer cells do not need to inactivate both *SAMHD1* alleles to increase the dNTP pools. Of note, the genes encoding Pol ε and δ are examples of two other genes in which heterozygous mutations have recently been associated with human cancers (58).

dNTP pool increases in *SAMHD1*$^{+/-}$ mouse embryos are modest but significant and range from 20% to 60% (Fig. 2). In fact, even modest changes of dNTP pools above or below normal levels can have a profound effect on cellular physiology or DNA replication fidelity. First, a mutation in the allosteric activity site of yeast RNR (*rnr1*-D57N) leads to only a ∼1.6- to 2-fold increase in dNTP pools, but a concomitant 3-fold increase in the mutation rate (11). Second, inactivation of Sml1, an inhibitor of yeast RNR, leads to a ∼2.5-fold increase in dNTP pools (59), and this slight increase is enough to rescue the lethality of the deletion of *MEC1* (yeast homolog of mammalian *ATR*) (59) and to increase the speed of replication forks by approximately 2-fold (60). Third, in mice, increased gene dosage of the small RNR subunit Rrm2 elevates RNR activity, but does not lead to elevated dNTP pools (61), presumably because of the strict allosteric dATP feedback inhibition of the mammalian RNR. Interestingly, despite any detectable increase in dNTP levels, increased gene dosage of the small RNR subunit Rrm2 reduces fragile site breakage and prolongs the survival of *ATR* mutant mice (61). Fourth, the deletion of Dun1, a protein kinase that controls yeast RNR, causes a ∼50% reduction of dNTP pools that, in turn, decreases the mutagenic effect of proofreading-deficient Pol ε (*pol2-4*) by approximately threefold and results in a mutation rate comparable to WT levels (18). Although the reduction of dNTP levels increases the fidelity of DNA polymerases, it can also lead to

fork stalling, the accumulation of single-stranded DNA, and chromosomal rearrangements. Consistent with these outcomes, decreased dNTP pools have been proposed to be a source of genomic instability in early stages of cancer development (62).

Cancer cells that have defects in replicative DNA polymerases and/or MMR will be at further risk of having elevated mutation rates due to minor dNTP pool alterations. We have recently demonstrated that the introduction of the Pol ε-M644G (a mutation that reduces the accuracy of Pol ε) or Pol δ-R696W (a human colon cancer-associated mutation) into budding yeast cells results in replication stress, leading to the activation of the genome integrity checkpoint and concomitant elevation of dNTP pools (17–19). The checkpoint-dependent elevation of dNTP pools was to a large degree responsible for the dramatic elevation of the mutation rates in these polymerase-defective yeast strains. Here, we show that minor increases of dNTP pools also have a profound effect on the mutation rate in an MMR-deficient yeast strain. The onefold to threefold increase in dNTP pools caused by the *mr1*-Y285F substitution elevates the mutation rate from $\sim 65 \times 10^{-7}$ in *msh2*Δ yeast to $\sim 200 \times 10^{-7}$ in *msh2*Δ *mr1*-Y285F yeast (Fig. 3*B*). To model the effect of minor dNTP pool alterations on the mutation rates in cancer cells, we perturbed dNTP pools in the HCT116 colorectal carcinoma cell line by adding low concentrations of thymidine and deoxyadenosine to the growth medium. Such treatment resulted in an approximately twofold increase in dTTP and dATP, an approximately twofold decrease in dCTP and dGTP, and a concomitant approximately threefold elevation of mutation rates at the *HPRT* locus. Because the HCT116 cells lack functional MMR, they already have ∼12-fold elevated mutation rates, compared with the HCT116 cells with normal MMR function (52). Our results indicate that minor alterations of dNTP pools further elevate mutation rates in MMR-deficient cells.

Replication stress-dependent activation of the genome integrity checkpoint has been shown to lead to elevated dNTP pools and to higher mutation rates in budding yeast (11). In contrast to yeast, genome integrity checkpoint activation in mammalian cells does not result in a similar global expansion of the dNTP pools (63). It is possible that, in response to checkpoint activation, there are local increases of dNTP levels near the sites of DNA damage. For example, colocalization of RNR and dTMP kinase at the sites of DNA damage has been reported (64, 65), but it is not known whether RNR and dTMP kinase colocalize at stalled replication forks. It is also not clear whether the rest of the machinery required for the production of dNTPs—including dUTPase, dCMP deaminase, dTMP synthase, dTMP kinase, and NDP kinase—colocalize at the sites of DNA damage. Even if there is no local checkpoint-dependent increase in dNTP pools in the mammalian cells in response to replication stress, it is possible that dNTP pools increase due to mutation or misregulation of one or more of the many enzymes involved in nucleotide metabolism.

In this study, we demonstrate that mutations in *SAMHD1* that alter its dNTPase activity are associated with colon cancer. We speculate that other mutations that elevate or imbalance dNTP pools will also be identified in cancer cells, and we propose that minor dNTP pool disturbances in combination with defects in proofreading or MMR might enhance the mutator phenotype of cancer cells. Recently, the minidriver model of polygenic cancer evolution has been put forward (66). This model proposes that many mutations found in cancer might not be major drivers or "passenger" mutations, but instead might have relatively weak tumor-promoting effects and are referred to as "mini drivers." It has been suggested that multiple mini drivers can substitute for a major driver. We believe that mutations in *SAMHD1* that are predicted to result in mutagenic dNTP pool alterations fall into the category of mini drivers.

The findings presented here might have implications for the treatment of mutator-driven cancers. Chemotherapeutic reduction of dNTP pools could decrease mutation rates and slow down cancer progression, whereas an additional elevation of dNTP pools by treatment with exogenous deoxyribonucleosides might further increase mutation rates and kill cancer cells through mutation overload.

Experimental Procedures

Public Databases. The cancer mutation database COSMIC was used to get an overview of reported SAMHD1 mutations in solid cancers. For detailed studies of tumors with SAMHD1 mutations, the publicly available mutation dataset (hgsc.bcm.edu COAD.IlluminaGA DNASeq.Level 2.1.5.0), consisting of data from 217 CRC tumors analyzed by using Illumina exome sequencing technology, was downloaded from TCGA. Only publicly available datasets were used for all analyses.

In Silico Analysis of Mutations. The deleteriousness of the *SAMHD1* mutations in the tumors of the large intestine from COSMIC was analyzed by using four different computational tools: Grantham, SIFT, PolyPhen2, and CADD. Because these are prediction tools, we increased our confidence in their results by taking into consideration the overlap between them when evaluating whether mutations were likely to affect protein function. ConSurf was used to generate conservation scores from 1 to 9, with 9 being the most conserved amino acids within the protein. The TCGA mutation dataset was analyzed by using the SMG test from the MuSIC suite with default settings in all cases except for background mutation rate groups, which was set to 2 based on the fact that colon cancers can be divided into hypermutated and non-hypermutated.

SAMHD1 Expression and Purification. The pGEX-6P-1 plasmids encoding N-terminal GST-tagged WT and mutant *SAMHD1* were expressed in *Escherichia coli* (BL21) grown in LB medium by using 0.4 mM isopropyl β-D-1-thio-galactopyranoside for induction at 18 °C for 16 h, and the proteins were purified essentially as described (67). After batch loading on 1.5 mL of glutathione–Sepharose (GE Healthcare), the beads were collected in a column and washed twice with 10 mL of buffer A [50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, and 10% (vol/vol) glycerol]. The GST affinity tag was removed by overnight enzymatic cleavage with PreScission Protease. SAMHD1 was eluted and stored in buffer A.

SAMHD1 in Vitro dNTPase Assay. dNTPase assays were performed essentially as described (68). Reactions in a total volume of 300 μL [10 mM Tris·HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM GTP, 1 mM dNTP, and 0.5 μM SAMHD1] were incubated at 25 °C. Aliquots collected at 0, 15, 30, 45, and 60 min were diluted in nine volumes of ice-cold PBS to stop the reaction and spun through a 0.5-mL Nanosep 3-kDa filter (PALL) at 14,000 × *g* for 10 min. The deproteinized samples were analyzed by HPLC using an UltraCore SuperC18 50-mm × 2.1-mm column (ACE) equilibrated in buffer (7% MeOH and 17 mM KH_2PO_4, pH 6.0). The dN products were quantified in buffer (7% MeOH and 17 mM KH_2PO_4, pH 6.0) by using the peak integration of the mV response. The specific activity of SAMHD1 was defined as nanomoles of dN product per hour (1 nmol of dN per hour = 1 unit) per milligram of protein [(nanomoles of dN per hour) per milligram].

dNTP Pool Measurements in Mouse Embryos. All animal experiments were approved by the Animal Review Board at the Court of Appeal of Northern Norrland (Umea). Homozygous *SAMHD1*$^{-/-}$ knockout mice in the C57BL/6 background (57) were kindly provided by Jan Rehwinkel (University of Oxford, Oxford) and mated with WT C57BL/6 mice. From these crosses, E13.5 embryos were isolated, and after their tails were removed for genotyping, the embryos were snap-frozen in Eppendorf tubes in liquid nitrogen. After the addition of ice-cold 12% (wt/vol) TCA, 15 mM $MgCl_2$ solution, and glass beads, the embryos were thawed on ice and homogenized on a BeadBeater (BioSpec) for 30 s at 4 °C in a cold room. The supernatant was collected by centrifugation at 14,000 × *g* for 5 min at 4 °C and processed as described (69).

dNTP Pool and Mutation Rate Measurements in *S. cerevisiae*. All yeast culturing was carried out at 30 °C in YPAD (1% yeast extract, 2% bacto-peptone, and 20 mg/L adenine) liquid cultures in a shaking incubator at 160 rpm. For plates, the YPAD contained 2% agar. dNTP pools were measured in asynchronous cultures as described (69). The canavanine resistance assay was used to calculate mutation rates as described (10).

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