dCTP pyrophosphohydrolase exhibits nucleic accumulation in multiple carcinomas

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Abstract

Nucleoside triphosphate pyrophosphohydrolase (NTP-PPase) functions as one of the mechanisms to guarantee the fidelity of DNA replication through the cleavage of non-canonical nucleotides into di- or monophosphates. Human NTP-PPase is poorly understood and investigated. In the present study, by using tissue microarrays with the paired cancer and adjacent regions, we found that with the prevalent expression of dCTP pyrophosphohydrolase (dCTPP1) in the cytosol and nucleus in tumors investigated, DCTPP1 was inclined to accumulate in the nucleus of cancer cells compared to the paired adjacent tissue cells in multiple carcinomas including lung, breast, liver, cervical, gastric and esophageus cancer. More significantly, the higher DCTPP1 expression in the nucleus of lung, gastric and esophageus cancer cells was associated with histological subtypes. The nucleic accumulation of DCTPP1 was apparently observed as well when tumor cell line MCF-7 was treated with H2O2 in vitro. Considering the roles of DCTPP1 on restricting the concentration of non-canonical nucleotides in the nucleus pool, accumulation of DCTPP1 in the nucleus of tumor cells might suffice for maintaining the proper DNA replication in order to fulfill the requirement for the survival and proliferation of tumor cells.

Introduction

Besides the essential role as precursors of DNA/RNA synthesis, free canonical nucleotides in living organisms play various roles to maintain physiological life activity, such as signal transmitters, molecular switches, coenzymes or energy carriers. Therefore, accumulation of non-canonical nucleotides, such as oxo- or methylated-deoxynucleoside triphosphates, in (d)NTPs pool is a prominent cause to reduce the fidelity of DNA replication, leading to the retardation of cell survival and proliferation. Non-canonical nucleotides are generated either as byproducts of cellular metabolism or by deamination or oxidation of bases in natural nucleotides. Incorporation of these non-canonical nucleotides during DNA replication results in the ambiguous base pairing and subsequent accumulation of mutations. The incorporated non-canonical nucleotides can be repaired by DNA repair machinery, such as mismatch repair (MMR) and base excision repair (BER). In addition, cells also develop elaborate enzymatic systems that maintain the purity of nucleotide pools. This is achieved by one class of enzymes named nucleoside triphosphate pyrophosphohydrolase (NTP-PPase) to eliminate the non-canonical nucleotides through cleaving them into di- or mono-phosphates. MutT-like proteins are the most extensively studied NTP-PPase which is involved in degrading oxidized purine nucleotides, such as 8-oxo-dGTP and 2-OH-dATP. A lack of mutT in Escherichia coli increases AT-CG mutation in both DNA and mRNA, while depletion of MTH1 in mice leads to a higher incidence of spontaneous tumors. The MazG family enzymes belong to all-alpha NTP-PPase including dimeric dUTPases, HisE and MazG, et al. It is supposed that they might perform house-cleaning function by degrading abnormal (d)NTPs, such as dUTP, 2-hydroxy-dATP and 8-oxo-dGTP, preventing them from incorporating into DNA or RNA. However, biological significance of MazG remains an enigma until recently. An essential role for MazG in larval molting and development is demonstrated in the parasitic roundworm Ascaris. In Caenorhabditis elegans, MazG is required for larval development and intestinal function. The study on MazG from Mycobacterium smegmatis demonstrates that it is required for full oxidative stress responses. The mouse RS21-C6 contains a typical conserved MazG domain. It can hydrolyze canonical dCTP into dCMP and prevent inappropriate DNA methylation, aberrant DNA replication or DNA mutagenesis by hydrolyzing modified dCTP. Human NTP-PPase is poorly understood and investigated. Inosine triphosphate pyrophosphohydrolase is proved to be pivotal in maintaining genome stability and preventing apoptosis in human cells. A few studies have reported the associations of NTP-PPase with hyperthyroidism and neoplasms. dCTP pyrophosphohydrolase (DCTPP1) is a newly defined MazG ortholog in human. It is speculated to specifically hydrolyze 5-methyl-2-deoxy-cytidine triphosphate, probably avoiding the incorporation of non-canonical nucleotides into mitotic DNA and the silence of functional genes. Due to its potential roles in modulating nucleic acid metabolism, it might be engaged in multiple metabolic disorders including tumorigenesis which is worthwhile further investigating.

In this study, we have primarily investigated the DCTTP1 expression profiles in normal and cancerous tissues. We found that DCTTP1 was significantly accumulated in the nucleus of liver, breast, lung, gastric, esophageus cancer cells as well as cervical carcinoma, which implies the potential roles of DCTPP1 under malignant pathology.

Materials and Methods

Tissue microarray
All the tissue microarrays (TMA) under...
mouse serum was used for the substitution of
in the IHC procedures, PBS with 10% normal
strate and counterstained with hematoxylin,
diaminobenzidine (DAB; Sigma-Aldrich) sub-
respectively. Slides were developed with
Sigma-Aldrich) at dilutions of 1:800 and 1:30,
conjugated horseradish peroxidase
1% goat serum, rabbit anti-human DCTPP1
retrieval. After blocking with PBS containing
0.01 M citrate buffer (pH 6.0) for antigen
microarray slides were deparaffinized and
fields were randomly selected. Total and nucle-
ic staining of DCTPP1 was scored by conven-
tional semi-quantitative scoring system. The
extent of staining was scored according to the
percentage of DCTPP1-positive cells as follows:
+ (1-24 %), ++ (25-49 %), +++ (50-74 %), or
++++ (75-100 %).14 respectively. For data
analysis, scored as + was considered low-
expression, other scores were considered high-expression.

In vitro treatment of MCF-7 with
H2O2
Human breast cancer cell line MCF-7 was
 Purchased for American Type Culture
Collection (Manassas, VA, USA) and main-
tained routinely in RPMI-1640 complete me-
dium with 10% fetal bovine serum (FBS) and 100
µg/mL penicillin-streptomycin (Invitrogen,
Carslbad, CA, USA). For in vitro treatment of
H2O2, MCF-7 cells were seeded on the cover
glasses in 24-well cell culture plate (5x10^4 cells
per well) in 500 µL RPMI-1640 complete me-
dium overnight. The cells were treated with H2O2
at concentration of 50 µM in complete medium
for 0, 1 and 2 h, respectively. After the treat-
ment, cells were washed with PBS and fixed in
pre-cold acetone for 5-10 min and subjected to
immunohistochemical staining against
DCTPP1 as described above.

Western blotting
MCF-7 cells with or without H2O2 treatment
were collected by trypsinization and spin. Cells
were lysed in lysis buffer [25 mM Tris–HCl pH
7.6, 150 mM NaCl, 1% NP-40, 1% sodium
dodecyl sulfate, 0.1% SDS, and 1% protease
inhibitor cocktail (Sigma-Aldrich)]. Protein
centrations were determined using the
Bio-Rad protein assay kit (Bio-Rad, Hercules,
CA, USA). The cell lysates were separated on
12% SDS-PAGE gels and transferred to a PVDF
membrane (Millipore, Billerica, MA, USA).
The membrane was incubated for 1 h at room
mperature in TBST solution [10 mM Tris–HCl
(pH 8.0), 150 mM NaCl, and 0.05% Tween-20]
supplemented with 5% nonfat dry milk and
probed overnight at 4°C with antibodies
against human DCTPP1 pAb (Abgent Inc., San
Diego, CA, USA) at dilutions of 1:500. Binding
antibodies were visualized using a suitable
secondary antibody conjugated to horseradish
peroxidase (1:2000, Sigma-Aldrich) and
enhanced chemiluminescence reagents
(Thermo, Rockford, IL, USA). Antibody against
tubulin (Sigma-Aldrich) was used to normalize
the quantity of the sample loading.

Statistical analysis
Analysis was performed with SPSS 16.0 soft-
ware package (SPSS Inc., Chicago, IL, USA).
The intergroup differences were examined by
using χ2-test or Fisher’s exact test. A P value
<0.05 was considered statistically significant.

Results
DCTPP1 expression in multiple
cancer tissue microarrays
To explore the possible role of DCTPP1 in
tumorigenesis, we first surveyed the DCTPP1
expression in the total regions of commercial
tissue microarrays with paired cancer and the
adjacent regions from multiple carcinomas. Tissue
microarrays including 89 cases of lung
cancer, 61 cases of breast cancer, 31 cases of
gastric cancer, 31 cases of esophagus cancer,
31 cases of liver cancer, 31 cases of cervical
cancer, and 29 cases of colorectal cancer
were under investigation in this study. For each
case, a paired adjacent tissue was studied in
parallel as control. Our results indicated that
DCTPP1 displayed cytosol and nucleic expres-
sing patterns in both cancer and adjacent
regions. Through statistical analysis, we found
that both in lung and breast cancer, DCTPP1
was significantly hyper-expressed in cancer
cells compared to the adjacent regions. In
other five types of cancer, the expression levels
of DCTPP1 were comparable between paired
cancer and adjacent regions (Table 1).

Higher nucleic DCTPP1 expressing
levels in cancer cells from multiple
carcinomas
During our study, a very interesting phe-
omenon was observed that DCTPP1 in most
of the cancer cells was highly expressed in the
nucleus when examined precisely in tissue
microarrays. However, in the adjacent regions
the nucleic expression level of DCTPP1 was
less apparent. Considering the fundamental
role of pyrophosphatase in controlling the
elimination of non-canonical nucleotides, it is
rational that the expression of DCTPP1 in the
nucleus is corresponding to its biological
function. Whether the nucleic DCTPP1 expression
is different between the cancer and the adja-
cent tissue cells was further studied in tissue
microarrays. Our results indicated that the
frequency of positive DCTPP1 staining in the
nucleus of six types of cancer cells was higher
than that in tissues adjacent to tumors (Table
2). For instance, in lung cancer tissue microaar-
ray three out of 89 tissues adjacent to the can-
cer cells showed high nucleic DCTPP1 stain-
ing. Among the 89 paired lung cancer tissues,
68 cases exhibited strong positive staining in
the nucleus and the rest 21 were weak for
DCTPP1 (Figure 1). As shown in Table 2, there

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were significant differences in terms of the number of cases with high nucleic DCTPP1 expression between the lung cancer and the adjacent group (76.4% vs 3.3%, P=0.000). In breast cancer, the high expression rate of nucleic DCTPP1 was 93.4% (57/61) in the cancer cells while it was only 54.1% (33/61) in the adjacent normal cells (Table 2; Figure 2), indicating dramatically difference of high nucleic DCTPP1 expression between the breast cancer and the adjacent controls (P=0.000). In liver carcinoma, 15 out of 31 tumor tissues were strong positive DCTPP1 staining in the nucleus whereas 1 out of 31 adjacent tissues displayed strong expression (Figure 2). The number of cases with high nucleic DCTPP1 expression between the liver cancer and the adjacent controls (48.4% vs 3.2%, P=0.000) was also dramatically different. Cervical cancer was another type of carcinoma that displayed significant difference of high nucleic DCTPP1 expression between cancer cells and the adjacent tissue cells (67.7% vs 25.8%, P<0.001) (Figure 2). The difference is not so dramatic but still significant between the gastric carcinoma and the paired adjacent controls (87.1% vs 61.3%, P=0.02) (Figure 3) as well as in esophagus carcinoma (67.7% vs 41.9%, P=0.048) (Figure 4). However, there was no significant difference of high nucleic DCTPP1 expression in colorectal cancer cells when compared to the paired adjacent cells (P=0.05) (Table 2). These results indicated that nucleic
d
Table 1. DCTPP1 expression in seven types of cancer and the paired adjacent regions in tissue microarrays.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Case No.</th>
<th>DCTPP1 expression</th>
<th>Paired adjacent region</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>89</td>
<td>Low: 7 (7.9%)</td>
<td>High: 82 (92.1%)</td>
<td>Low: 22 (24.7%)</td>
</tr>
<tr>
<td>Breast</td>
<td>61</td>
<td>Low: 5 (8.2%)</td>
<td>High: 56 (91.8%)</td>
<td>Low: 34 (55.7%)</td>
</tr>
<tr>
<td>Liver</td>
<td>31</td>
<td>Low: 1 (3.2%)</td>
<td>High: 30 (96.8%)</td>
<td>Low: 2 (6.5%)</td>
</tr>
<tr>
<td>Cervical</td>
<td>31</td>
<td>Low: 2 (6.5%)</td>
<td>High: 29 (93.5%)</td>
<td>Low: 4 (12.9%)</td>
</tr>
<tr>
<td>Stomach</td>
<td>31</td>
<td>Low: 5 (16.1%)</td>
<td>High: 26 (83.9%)</td>
<td>Low: 5 (16.1%)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>31</td>
<td>Low: 2 (6.5%)</td>
<td>High: 29 (93.5%)</td>
<td>Low: 3 (9.7%)</td>
</tr>
<tr>
<td>Colon</td>
<td>29</td>
<td>Low: 2 (6.9%)</td>
<td>High: 27 (93.1%)</td>
<td>Low: 3 (10.3%)</td>
</tr>
</tbody>
</table>

*High expression of DCTPP1 in cancer region vs the paired adjacent region. The high expression of DCTPP1 is defined as the percentage of DCTPP1-positive cells more than 25% observed.

Table 2. Nucleic DCTPP1 expression in seven types of cancer tissues and the paired adjacent regions.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Case No.</th>
<th>Nucleic DCTPP1 expression</th>
<th>Paired adjacent region</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>89</td>
<td>Low: 21 (23.6%)</td>
<td>High: 68 (76.4%)</td>
<td>Low: 86 (96.6%)</td>
</tr>
<tr>
<td>Breast</td>
<td>61</td>
<td>Low: 4 (6.6%)</td>
<td>High: 57 (93.4%)</td>
<td>Low: 28 (45.9%)</td>
</tr>
<tr>
<td>Liver</td>
<td>31</td>
<td>Low: 16 (51.6%)</td>
<td>High: 15 (48.4%)</td>
<td>Low: 30 (96.8%)</td>
</tr>
<tr>
<td>Cervical</td>
<td>31</td>
<td>Low: 10 (32.3%)</td>
<td>High: 21 (67.7%)</td>
<td>Low: 23 (74.2%)</td>
</tr>
<tr>
<td>Stomach</td>
<td>31</td>
<td>Low: 4 (12.9%)</td>
<td>High: 27 (87.1%)</td>
<td>Low: 12 (38.7%)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>31</td>
<td>Low: 10 (32.3%)</td>
<td>High: 21 (67.7%)</td>
<td>Low: 18 (58.1%)</td>
</tr>
<tr>
<td>Colon</td>
<td>29</td>
<td>Low: 4 (13.8%)</td>
<td>High: 25 (86.2%)</td>
<td>Low: 10 (34.5%)</td>
</tr>
</tbody>
</table>

*High expression of DCTPP1 in cancer region vs the paired adjacent region. The high expression of DCTPP1 is defined as the percentage of DCTPP1-positive cells more than 25% observed.

Table 3. Association of clinicopathological features of lung cancer, gastric cancer and esophagus cancer with nucleic DCTPP1 expression.

<table>
<thead>
<tr>
<th>Clinicopathologic parameters</th>
<th>Nucleic DCTPP1 expression (%)</th>
<th>Gastric cancer expression (%)</th>
<th>Esophagus cancer expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>Case No. Low High P</td>
<td>Case No. Low High P</td>
<td>Case No. Low High P</td>
</tr>
<tr>
<td>Age &lt;60</td>
<td>32 7(21.9%) 25(78.1%) 0.768</td>
<td>15 2(13.3%) 13(86.7%) 0.613</td>
<td>18 7(38.9%) 11(61.1%) 0.694</td>
</tr>
<tr>
<td>≥60</td>
<td>57 14(24.6%) 43(75.4%)</td>
<td>16 3(18.8%) 13(81.2%)</td>
<td>13 3(23.1%) 10(76.9%)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 69 16(23.2%) 53(76.8%) 0.775</td>
<td>21 2(9.5%) 19(90.5%) 0.577</td>
<td>27 9(33.3%) 18(66.7%) 1.000</td>
</tr>
<tr>
<td>Female</td>
<td>20 5(25.0%) 15(75.0%)</td>
<td>10 2(20.0%) 8(80.0%)</td>
<td>4 1(25.0%) 3(75.0%)</td>
</tr>
<tr>
<td>Histological subtype</td>
<td>SCLC 8 0(0%) 8(100%) 0.190</td>
<td>SC 6 3(50.0%) 3(50.0%)</td>
<td>AC 18 4(44.4%) 5(55.6%) 0.032</td>
</tr>
<tr>
<td>LCC (NSCLC)</td>
<td>10 2(20.0%) 8(80.0%) 0.016</td>
<td>12 1(8.3%) 11(91.7%) 0.023</td>
<td>15 2(13.3%) 13(86.7%) 0.032</td>
</tr>
<tr>
<td>SC (NSCLC)</td>
<td>41 15(36.6%) 26(63.4%) 0.015</td>
<td>UC 6 0(0%) 6(100%) 0.032</td>
<td>SCC 6 4(66.7%) 2(33.3%) 0.048</td>
</tr>
<tr>
<td>SAC (NSCLC)</td>
<td>10 1(10.0%) 9(90.0%) 0.015</td>
<td>SR 7 0(0%) 7(100%) 0.032</td>
<td>UC 1 0(0%) 1(100%)</td>
</tr>
<tr>
<td>AC (NSCLC)</td>
<td>20 3(15.0%) 17(85.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SCLC, small cell lung cancer; NSCLC, non small cell lung cancer; LCC, large cell carcinoma; SC, squamous carcinoma; SAC, squamous adeno carcinoma; AC, adenocarcinoma; SCC, small cell cancer; UC, undifferentiated carcinoma, SR, signet ring cell carcinoma. *Compared between SCLC and NSCLC; **compared among subtypes of NSCLC.
expression of DCTPPI was more remarkable in cancer cells although the overall expression level of DCTPPI was similar in cancer and the paired adjacent tissue regions.

Association of nucleic DCTPPI expression with clinicopathologic features

To further evaluate the significance of DCTPPI in tumor, the clinical, epidemiologic, and histopathologic characteristics of patients with lung cancer, gastric cancer and esophageal cancer, and the association of high nucleic expression of DCTPPI with the clinicopathologic parameters were analyzed. As shown in Table 3, there were no statistically significant associations in the high nucleic expression of DCTPPI with regard to patients’ age and gender. However, the correlation between histological subtypes and the levels of DCTPPI in non-small cell lung cancer (P=0.016), gastric cancer (P=0.023) or esophageus cancer (P=0.032) were statistically striking. For instance, in gastric cancer patients, the nucleic DCTPPI expression was significantly correlated with histological subtypes. DCTPPI expression was higher in signet ring cell carcinoma (77%) and undifferentiated carcinoma (66%) than in tubular adenocarcinoma (11/12) or squamous carcinoma (3/6). In esophageus cancer tissue samples, the percentages of high nucleic DCTPPI expression in adenocarcinoma, squamous carcinoma and small cell cancer patients were 91.7% (11/12), 55.6% (5/9) and 33.3% (2/6), respectively. In lung cancer, the nucleic DCTPPI was highly expressed in all small cell lung cancer (SCLC) patients (10/10) whereas only 60 out of 81 non-SCLC patients displayed high DCTPPI nucleic expression.

When we subgrouped the non-SCLC patients into squamous carcinoma, adenocarcinoma, squamous adenocarcinoma and large cell carcinoma subtypes, the expressing rate of high DCTPPI expression in the nucleus was lowest in squamous carcinoma patients (26/41) whereas comparable among other three groups. In addition, no strong correlations in the nucleic accumulation of DCTPPI with clinicopathology were detectable in breast cancer, liver cancer, colon cancer and cervical cancer patients (data not shown).

Nucleic accumulation of DCTPPI upon H₂O₂ treatment in vitro

To further investigate the feasibility of DCTPPI nucleic accumulation, a breast cancer cell line MCF-7 was subjected to be stimulated with H₂O₂ for different duration. Expression of DCTPPI was determined by immunohistochemical staining and western blotting. As shown in Figure 5A, DCTPPI expression was up-regulated in MCF-7 upon H₂O₂ stimulation after 1-hour-treatment. When looking closely at the distribution of DCTPPI in MCF-7 cells, we observed that DCTPPI molecules started to accumulate in the nucleus after one hour’s treatment (Figure 5 B-c) and the tendency was more apparent at 2 h (Figure 5 B-d), which demonstrated the occurrence of nucleic accumulation of DCTPPI when encountering extra stress signals.

Discussion

In the present study we have, for the first time, explored the expression pattern of DCTPPI in multiple carcinomas. Our results revealed the hyper-expression property in the nucleus, suggesting nucleus accumulation of DCTPPI in cancerous tissues compared to the corresponding adjacent tissues investigated.

NTP-PPase is supposed to play an important role in regulating DNA replication and energy metabolism. Members of the NTP-PPase family have been shown to eliminate non-canonical nucleotides from the intracellular NTP pools. For example, dUTPase hydrolyzes deoxyuridine triphosphate while MTH1 hydrolyzes oxidized purine nucleotide triphosphates, including 8-oxo-(deoxy) guanosine triphosphate (8-oxo-(d)GTP) and 2-hydroxy-(deoxy) adenosine triphosphate (2-OH-(d)ATP). In cancer cells, the elevated NTP-PPase activity will probably...
lower the concentration of non-canonical nucleotides, prevent their aberrant incorporations into DNA and thus might make the cancer cells more resistance to DNA damage and apoptosis. Indeed, the overexpression of certain pyrophosphatases has recently been reported in gastric cancer,14 prostate cancer,15 hepatocellular carcinoma20 and brain cancer.21 In the present study, we have surveyed the prevalent expression of DCTPP1, a dCTP pyrophosphohydrolase in multiple carcinomas. Consistent with above mentioned previous studies, we have also observed the hyper-expression in lung and breast cancer, which provides another evidence to support the potential involvement of NTP-PPase in tumor.

Nucleic accumulation of DCTPP1 in cancer cells described here is another striking phenomenon. Nucleic translocation of molecules such as transcription factors from cytosol or membrane is mostly associated with cell proliferation and differentiation upon stress, immune stimuli as well as danger signals.22,23 In tumorigenesis, many tumor-associated molecules including p53,24 HER-2,25 androgen receptor,26 nuclear transcription factors like NF-kB27 etc., are reported to perform the aberrant nucleic translocation which are demonstrated to be critical for the proliferation, mitosis and transformation of tumor cells. In the present study, nucleic accumulation of DCTPP1 in cancer cells investigated clearly meets its putative functionality in guaranteeing the correct DNA synthesis under mitosis of tumor cells. To our knowledge, this is the first report describing the association between NTP-PPase nucleus distribution and tumor. Our further study indicates that under oxidative stress DCTPP1 accumulates in the nucleus which is consistent with its house-cleaning function. Although it is not exactly the same situation compared to tumorigenesis or progression, we still speculate that DCTPP1 overexpression in the nucleus meets the requirement for tumorigenesis through adjusting the nucleotide metabolism with less non-canonical nucleotide concentration in the nucleotide pool. It needs further investigation to understand why and how DCTPP1 accumulates into the nucleus under pathological circumstances.

In this study, there was no significant difference of high nucleic DCTPP1 expression in colorectal cancer cells when compared to the paired adjacent tissue cells. One of the possibilities is due to the small numbers of colorectal tissue samples in tissue microarrays we have investigated (n=29). In addition, it is necessary to classify the samples according to histopathology. In fact, we have found that the nucleic DCTPP1 was significantly overexpressed in the adenocarcinoma than in squamous carcinoma (P<0.05) in our study (data not shown). According to our study, DCTPP1 expression is diverse in different histological subtypes of lung, gastric and esophageal cancer. Although it is hard to bridge the malignancy and the histological subtypes in clinic, one of the key orientations of histological subtyping lies in the determination of chemotherapy strategy for the patients. For instance, in lung cancer, small cell lung cancer (SCLC) is more sensitive to chemotherapy than non-small cell lung cancer (NSCLC). Among NSCLC, patients with squamous, adenocarcinoma or squamous adenocarcinoma lung cancer also undergo diverse clinical chemotherapy combination. Our discovery on the association of DCTPP1 with histological subtypes thus raises another possibility that with the different expression levels of DCTPP1 in tumors of different histological subtypes, DCTPP1 might be a potential new indicator to evaluate the efficacy of chemo-sensitivity against tumor. If so, to develop chemical compounds targeting DCTPP1 activity will probably improve the anti-tumor chemotherapy. In fact, it has already been reported that the inhibition of human deoxyuridine triphosphatase (dUTPase) has become a promising approach to enhance the efficacy of 5-fluouracil (5-FU)-based chemotherapy in vivo.28 In the future, molecular dissection of DCTPP1 in tumorigenesis and progression will accelerate the potential application of DCTPP1 as a novel target for cancer therapy.

What the biological role of DCTPP1 is in mammalian cells remains poorly understood. In normal cells, DCTPP1 is speculated to catalyse the hydrolysis of dCTP to dCMP in order to

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**Figure 3.** Representative examples of immunohistochemical staining of DCTPP1 in gastric cancer with different histological subtypes. DCTPP1 staining was determined simultaneously in tissue arrays with cancer and the paired adjacent tissue regions. Positive staining of DCTPP1 was shown in dark brown while the nucleus is stained with H&E dye. In chronic superficial gastritis tissue (A, C, E and G), the expression of DCTPP1 was slight both in the cytosol and nucleus. In the paired cancer tissues, such as: B) squamous cell carcinoma; D) adenocarcinoma; F) signet ring cell carcinoma; H) undifferentiated carcinoma, DCTPP1 was found highly expressed in the nucleus of cancer cells. Magnification: 400×; scale bar: 25 µm.

**Figure 4.** Representative examples of immunohistochemical staining of DCTPP1 in esophageal cancer with different histological subtypes. DCTPP1 staining was determined simultaneously in tissue microarrays with cancer and the paired adjacent tissue regions. Positive staining of DCTPP1 is shown in dark brown while nucleus is stained with H&E dye. In the paired adjacent tissues (A, C, E and G), the expression of DCTPP1 was apparent both in the cytosol and the nucleus. In squamous cell carcinoma(B), adenocarcinoma (D), small cell cancer (F) and undifferentiated carcinoma (H), DCTPP1 was found highly expressed in the nucleus of cancer cells. Magnification: 400×; scale bar: 25 µm.
maintain the dCMP pool at a certain level for thymidylate synthesis.14 dCMP is the main precursor of dTMP, which is converted to dTTP subsequently by a two-step phosphorylation reaction.29,30 It is reported that an imbalanced dCTP/dTTP ratio in hamster fibroblasts mildly affect the fidelity of DNA replication.31 To maintain the proper ratio of dCTP/dTTP in the nucleotide pool seems important for physiological homeostasis. The activity of DCTPP1 in cells might, to some extent, be engaged in the modulation of the dCTP concentration in the nucleotide pool, which finally affects the ratio of dCTP/dTTP in the intracellular nucleotide pool.

More interesting is the biological function of DCTPP1 to prevent non-canonical 5-methyl-dCTP from the incorporation into newly synthesized DNA. This might be linked to the regulation of gene expression by dCTP methylation level. It has been reported that inflammation-mediated halogenated cytosine damage products can mimic 5-methyl-cytosine in directing enzymatic DNA methylation and enhancing the binding to methyl-binding proteins.32 The overwhelming 5-halogenated dCTP generated in chronic inflammatory tissues might be incorporated into promoter regions of important genes, like tumor-suppressor genes, inducing their silencing through inappropriate CpG methylation of the promoter regions or by binding to methyl-CpG-binding protein 2 and resulting in the transformation of normal tissue cells. In our study, we have found that nucleic DCTPP1 was also overexpressed in the inflammatory sites in gastric cancer and colorectal cancer (Figures 2G and 3A,E). We therefore propose that the nucleic expression of DCTPP1 to hydrolyze the modified deoxynucleotides might be one of the mechanisms to protect normal cells from the malignant transformation through degradation of cytosine damage products under inflammation.

In conclusion, the findings presented here indicate that aberrant nucleic DCTPP1 accumulation represents the common feature in cancers, suggesting the role of DCTPP1 in regulating the nucleotide metabolism while displaying its potential application in diagnosis and therapy in the future.

References


