Experimental and Molecular Pathology 99 (2015) 56–64

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Correlation between topoisomerase I and tyrosyl-DNA phosphodiesterase 1 activities in non-small cell lung cancer tissue

Ann-Katrine Jakobsen [a], Kristina Lystlund Lauridsen [a], Evelyn Benuja Samuel [a], Joanna Proszek [a], Birgitta Ruth Knudsen [b,c], Henrik Hager [a,d], Magnus Stougaard [a,*]

[a] Department of Pathology, Aarhus University Hospital, Denmark
[b] Department of Molecular Biology and Genetics, Aarhus University, Denmark
[c] Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark
[d] Department of Clinical Pathology, Vejle Hospital, Denmark

ARTICLE INFO

Article history:
Received 23 January 2015
and in revised form 4 May 2015
Accepted 14 May 2015
Available online 16 May 2015

Keywords:
Lung cancer
Topoisomerase I
Tyrosyl-DNA phosphodiesterase 1
Biosensor
Cryosection

ABSTRACT

Topoisomerase I (TOP1) regulates DNA topology during replication and transcription whereas tyrosyl-DNA phosphodiesterase 1 (TDP1) is involved in the repair of several types of DNA damages, including damages from defective TOP1 catalysis. TOP1 is the target of chemotherapeutic drugs of the camptothecin family (CPT). TDP1 has in cell line based assays been shown to counteract the effect of CPT.

We have quantified the enzymatic activities of TOP1 and TDP1 in paired (tumor and adjacent non-tumor) samples from non-small cell lung cancer (NSCLC) patients and show that in NSCLC TOP1 and TDP1 activities are significantly upregulated in the tumor tissue. Furthermore, we found a positive correlation between the TDP1 activity and the tumor percentage (TOP1 activity did not correlate with the tumor percentage) as well as between the activities of TOP1 and TDP1 both within the tumor and the non-tumor group.

That TDP1 activity was upregulated in all tumor samples and correlated with the tumor percentage suggest that it must play a highly important function in NSCLC. This could be to protect against TOP1 mediated DNA damage as the activity of TOP1 likewise was upregulated in the majority of tumor samples and correlated positively to the TDP1 activity. Regardless, the finding that the TOP1 and TDP1 activities are upregulated and correlate positively suggests that combinatorial treatment targeting both activities could be advantageous in NSCLC.

1. Introduction

Molecular characterization of individual cancers is becoming increasingly used in patient treatment. However, most analyses are based on detection of mutations (e.g. KRAS or EGFR Roberts and Stinchcombe, 2013), promoter methylations (MLH1 or MGMT Do et al., 2014), or genomic rearrangements (EML4-ALK Steuer and Ramalingam, 2014). Although these analyses are known to be valuable prognostic and/or predictive markers and have the benefit of not requiring fresh or frozen tissue samples they are indirect markers and do not measure the actual biological relevant activities. In some cases this can be a problem for example if using the clinical relevant anticancer drugs of the camptothecin (CPT) family such as irinotecan and topotecan. These drugs target topoisomerase I (TOP1) activity specifically and function by converting intracellular TOP1 activity into DNA damage ultimately killing the cell (Eng et al., 1988; Pommier, 2006) and are mainly used in systemic treatment of colon-, ovarian-, and small cell lung cancer (SCLC) (Fruh et al., 2013; Hartwell et al., 2011; Kalemkerian et al., 2013; Naumann and Coleman, 2011; Stein and Arnold, 2012). Consistently, high TOP1 activity has indeed been shown to correlate better with high CPT sensitivity than TOP1 gene amplification, increased mRNA amount, or increased protein amount (Goldwasser et al., 1995; Jansen et al., 1997; Proszek et al., 2013). The better correlation could likely be due to post-translational modification of TOP1 (Bandyopadhyay and Gjerset, 2011; Roy et al., 2014) influencing the activity. Despite this knowledge, no preselection of patients with regard to TOP1 activity status is currently performed before CPT based treatment is commenced.

TOP1 is an essential nuclear enzyme important for the release of topological stress introduced during processes such as transcription and replication, where the DNA double helix is locally unwound. Its catalytic activity involves the transient cleavage of one strand in the DNA double helix causing TOP1 to be covalently attached to the DNA, release of supercoils through rotation of the cut strand around the scissile strand, and subsequent religation and release of the DNA (Champoux, 2001). CPT inhibits the religation step of the catalysis transiently trapping TOP1 covalently attached in a TOP1-DNA complex. In dividing cells such transiently trapped cleavage complexes can result in the generation of permanent double-stranded DNA breaks by S-phase specific collision with the replication machinery (D'Arpa et al., 1990). Besides drugs of the camptothecin family TOP1-DNA complexes may also be stabilized by naturally occurring DNA lesions such as nicks, abasic sites

http://dx.doi.org/10.1016/j.yexmp.2015.05.006
0014-4800/

or mismatches. As a consequence, cells possess repair mechanisms capable of removing such complexes before they become toxic and one of the key enzymes in this repair is tyrosyl-DNA phosphodiesterase 1 (TDP1) (Pourquier et al., 1997a, 1997b).

TDP1 is part of the X-ray repair complementation group 1 (XRCC1) complex which interacts with poly (ADP-ribose) polymerase (PARP) (Das et al., 2014; Plo et al., 2003) and was initially discovered by its ability to remove TOP1 covalently trapped to the DNA by cleaving the 3′ phosphotyrosyl linkage in TOP1–DNA complexes (Yang et al., 1996). In human cells TDP1 is required for repair of chromosomal single-strand breaks arising independently of DNA replication from abortive TOP1 activity or oxidative stress (El-Khamisy et al., 2005). Furthermore, studies have shown that TDP1 can remove several different moieties from the 3′end of DNA (Huang et al., 2013; Inamdar et al., 2002; Interthal et al., 2005a; Jensen et al., 2013) suggesting the importance of TDP1 for the repair of a number of DNA lesions other than TOP1–DNA complexes (Ben Hassine and Arcangioli, 2009; Das et al., 2010; Zhou et al., 2005). A homozygous mutation in TDP1 (A1478G, changing histidine 493 to arginine) has been identified as the sole cause of the disease, spinocerebellar ataxia with axonal neuropathy (SCAN1) (Takashima et al., 2002). This mutation results in a TDP1 enzyme with greatly reduced activity and the accumulation of TDP1–DNA complexes (Interthal et al., 2005b). The mutation does not predispose to neoplasia or dysfunctions in rapidly replicating tissues (Takashima et al., 2002), probably due to different enzymes complementing the function of TDP1 in normally functioning cells (Dexheimer et al., 2008; Liu et al., 2002; Zhang et al., 2011). However, high TDP1 activity may counteract CPT induced DNA damage (Barthelmes et al., 2004; Nivens et al., 2004).

CPT derivatives have, besides the use in treatment of SCLC, been suggested as viable treatment options in advanced non-small cell lung cancer (NSCLC) (Chen et al., 2012; O'Brien et al., 2007; Tsakalozou et al., 2014). However, information concerning enzymatic activities that can affect CPT-based therapy is scarce. TOP1 and TDP1 are ubiquitously expressed in several human tissues (Fam et al., 2013; Husain et al., 1994), and the expressions are upregulated in non-small cell lung cancer (NSCLC) (Giaccone et al., 1995; Liu et al., 2007). Furthermore, TDP1 activity has been shown to be upregulated although the protein amount was not increased in NSCLC (Liu et al., 2007) exemplifying the relevance of testing for activity.

Using our previously developed nanosensors capable of measuring the specific activities of TOP1 (Stougaard et al., 2009) and TDP1 (Jensen et al., 2013) we examined the activities of TOP1 and TDP1 in cryosections from 24 paired (tumor and adjacent non-tumor) NSCLC tissues. We found that both TOP1 and TDP1 were upregulated in the tumor tissue compared to the adjacent non-tumor tissue in NSCLC. Despite the limited amount of samples included in this study we saw a positive correlation between the percentage of tumor cells and TDP1 activity. No correlation was found between TOP1 and the percentage of tumor cells. Lastly, we found a positive correlation between the activities of TOP1 and TDP1 suggesting that combinatorial treatment targeting both TOP1 and TDP1 could be desirable.

2. Material and methods

2.1. Synthetic DNA oligonucleotides and chemicals

All oligonucleotides were obtained from DNA Technology A/S (Aarhus, Denmark) except the TOP1 nanosensor, TOP1-Id16, which was obtained from Sigma Aldrich (Broenby, Denmark). CodeLink Activated Slides came from SurModics (Eden Prairie, MN, USA), and Vectashield was from Vector Laboratories (Burlinghame, CA, USA). Pap Pen was purchased from Dako (Glostrup, Denmark). Phi29 DNA polymerase was from Thermo Scientific. Tissue-Tek OCT compound was purchased from Sakura Finetek (Copenhagen S, Denmark).

The TDP1 nanosensor had the sequence 5′-ATTO488-phosphothioate-AAA GCA GGC TTC AAC GCA ACT GTG AAG ATC GCT TGG GTG CGT TGA AGC CTG CTT T-BHQ1-3′. The TOP1 nanosensor, TOP1-Id16, had the sequence 5′-AGA AAA ATT TTT AAA AAA ACT GTG AAG ATC GCT TAT TTT TTT AAA AAT TTT TCT AAG TCT TTT AGA TCC CTC AAT GCT GCT GCT GTA CTA CGA TCT AAA AGA CTT AGA-3′, the positive control circle, PosC-IdA1, had the sequence 5′-p-AGA AAA ATT TTT AAA AAA ACT GTG AAG ATC GCT TAT TTT TTT AAA AAT TTT TCT AAG TCT TTT AGA TCC CGA GAT GTA CCG CTA TCG TGA TCT AAA AGA CTT-3′, the fluorescently labeled detection oligonucleotides, ID16-TAMRA and IDA1-6FAM, had the sequences 5′-TAMRA-CCT CAA TGC TGC TGC TGT ACT AC-3′ and 5′-6FAM-CCG AGA TGT ACC GCT ATC GT-3′ respectively. The Rolling Circle Amplification (RCA) primer used for the TOP1 activity assay had the sequence 5′-C6amine-CCA ACC AAC CAA CCA AAT AAG CGA TCT TCA CAG T-3′.

2.2. Tissue samples, extract preparation, and measurement of area and protein concentration

This study was conducted using anonymous NSCLC tissue samples collected at Aarhus University Hospital in 2011 after approval by The Regional Committee on Health Research Ethics. The study included 24 paired tissue samples (one tumor and one adjacent non-tumor sample from each patient) embedded in Tissue-Tek OCT compound and stored at −80 °C. Tissue sections were cut using a Microm HM 560 cryostat. The area was measured by digitalizing the slides using HAMAMATSU Nanozoomer 2.0-HT scanner equipped with a 40× objective, imported into NDP view where the tissue area was manually marked and the area automatically calculated. The protein concentration was measured using a LabelGuard Microliter Cell and an Implen NanoPhotometer. Tissue sections were cut with a thickness of 5 or 10 μm as specified in each experiment, subsequently lysed in 60 μL 1× TDP1-buffer (20 mM Tris–HCl pH 8, 100 mM KCl, 10 mM DTT, 10 mM EDTA and 0.05% Triton X-100) and left on ice for 10 min. Calculation of the final concentration of OCT compound in the tissue extract was calculated from the formula, $V = \pi * r^2$ where the radius (r) of the tube was 6.5 mm and the thickness (h) of the cryosections varied from 5 μm, 10 μm, 20 μm (2 × 10 μm), or 30 μm (3 × 10 μm) and the total volume of the reaction was 60 μL. Average size of the tissue samples was 26.7 mm^2, thus the average radius was 2.95 mm.

2.3. Statistics

The number of patients included is specified for each analysis since missing data resulted in the exclusion of individual samples. The D'Agostino & Pearson omnibus test was used to test if the data was normally distributed and thus whether a parametric or non-parametric test should be used. The hypothesis of no difference between either the area ($n = 21$) or protein concentration ($n = 22$) in the tumor and non-tumor groups was tested using a two tailed unpaired t-test. The hypothesis of no difference between the enzymatic activity (TOP1 $n = 18$ and TDP1 $n = 22$) in the tumor and non-tumor groups was tested using a two tailed Mann Whitney. The hypothesis of independency between the activity of enzymatic activity (TOP1 $n = 16$ and TDP1 $n = 20$) and tumor percentage was tested using a two-tailed Spearman correlation test. The hypothesis of independency between the activity of TOP1 and TDP1 was tested using a two-tailed Spearman correlation test ($n = 18$). P-values < 0.05 were considered significant. All statistical analyses were performed with Graph Pad Prism (graphpad.com).

2.4. TDP1 activity measurement

The TDP1 activity assay was carried out essentially as described in Jensen et al. (2013). To test the influence of Tissue-Tek on the assay, the reaction was carried out in a final volume of 50 μL containing 1 μL purified TDP1 enzyme, 1× TDP1-buffer, 0.1 μM TDP1-biosensor, and different concentrations of Tissue-Tek (0%, 0.625%, 1.25%, 2.5%, 5% and 10%). The assays with tissue extract were carried out in a final volume

of 25 μL containing 22.5 μL tissue extract and 1 μM TDP1-biosensor. Real-time fluorescence measurements were performed at 37 °C in a qPCR machine measuring every 1 min for 2 h, whereupon the data was transferred to Microsoft Excel. The initial linear slope from the data was calculated for all samples and normalized to the measured OD of the extract and visualized as a XY-plot using GraphPad Prism.

2.5. TOP1 activity measurement

The TOP1 activity assay was carried out essentially as described in Stougaard et al. (Stougaard et al., 2009). For testing the influence of Tissue-Tek on the assay, the reaction was performed in 1× TDP1-buffer supplemented with 1 μL purified TOP1, 0.2 μM TOP1-Id16, and different concentrations of Tissue-Tek (0%, 0.625%, 1.25%, 2.5%, 5%, and 10%). The assays with tissue extract contained varying thicknesses of cryosections dissolved in 10 μL 1× TDP1-buffer tissue extract supplemented with 0.2 μM TOP1-Id16. Both types of reaction mixture were incubated for 30 min at 37 °C, followed by heat inactivating of the TOP1 enzyme at 95 °C for 5 min. To allow quantification, 0.5 μL of ligated 100 nM PosC-IdA1 was added to 5 μL of the heat inactivated reaction mixture, and NaCl was added to a final concentration of 250 mM to increase the efficiency of the hybridization. The 5′-amine-coupled RCA primer was linked to CodeLink Activated Slides according to the manufacturer's protocol. Subsequently, the now 5.5 μL of reaction mixture was hybridized to the surface coupled primer for 60 min at 37 °C. Slides were washed for 1 min at room temperature in wash buffer 1 (100 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.3% SDS) and subsequently for 1 min at room temperature in wash buffer 2 (100 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween-20). Finally, the slides were dehydrated in 99% ethanol for 1 min and air-dried. RCA was performed for 60 min at 37 °C in 3 μL mixture containing 1xPhi29 buffer supplemented with 0.2 μg/μL BSA, 250 μM dNTP, and 1 unit/μL Phi29 DNA polymerase. The reaction was stopped by washing in wash buffers 1 and 2 for 1 min each followed by dehydration in 99% ethanol for 1 min. The RCA products were detected by hybridization of 0.2 μM of each of the detection probes, ID16-TAMRA (red fluorescent labeled) and IDA1-6′FAM (green fluorescent labeled), in a buffer containing 20% formamide, 2× SSC, and 5% glycerol for 30 min at 37 °C in a volume of 5 μL. The slides were washed in wash buffer 1 for 10 min, in wash buffer 2 for 5 min, dehydrated in 99% ethanol for 1 min, mounted with Vectashield, and visualized under a fluorescence microscope. For quantitative depiction of the results, the number of signals originating from TOP1 activity (red signals) or spike in control circles (green signals), respectively, was counted on 10 pictures, and the relative TOP1 activity calculated as the ratio between the number of red (R) and green (G) signals (R/G) was normalized to the measured OD of the extract and visualized as a XY-plot using GraphPad Prism.

3. Results

3.1. Sensitivity of the nanosensors in heterogeneous tissue extract

There are several challenges when using nanosensors in clinical biopsies e.g. due to the sensitivity of the nanosensor, cellular heterogeneity in the biopsies, and percentage of tumor cells (Stougaard and Ho, 2013). The method used for measuring the enzyme activities is illustrated in Fig. 1. For each tissue sample 3–4 sections were cut, and the first and last were mounted on microscopic slides, hematoxylin and eosin (HE) stained, and used for estimation of the tumor percentage and tissue area. The middle section (s) was collected in a tube and used for measuring protein content and enzyme activity (Fig. 1).

Tissues such as lung may be highly heterogenic both with regard to differences in the percentage of tumor cells and to the overall cell density. This may influence the measurement and normalization to total protein in the extract is therefore preferred over size or weight. To ensure that the individual protein concentration measurements used for normalization was not biased by a higher overall protein expression in the tumor samples, we compared the protein concentration between the two groups (tumor and non-tumor) and found no statistical difference ($p = 0.3967$, see Supplementary Fig. 1). Consequently, to enable comparison of paired non-tumor and tumor samples (i.e. from the same patient) where up to a sevenfold difference in the protein concentration was found, all subsequent measurements were normalized to the protein concentration of the specific sample. To test the sensitivity of the TDP1 and TOP1 nanosensors in cryosections from the NSCLC tissue, we performed activity measurements in extract from sections corresponding to a thickness of 5 μm, 10 μm, 20 μm (2 × 10 μm), and 30 μm (3 × 10 μm) (Fig. 2A and B). Both the TOP1 nanosensor and TDP1 nanosensor allowed precise measurements down to 5 μm as illustrated from the linearity (Fig. 2A and B).

Embedding of tissue in OCT compound is performed by adding OCT compound into a tube and subsequently submerging the tissue into the OCT compound. Thus, larger pieces of tissue will displace more OCT than small pieces inevitably resulting in varying concentration of OCT compound in the tissue extract which could influence the measurements. Although there was up to sevenfold difference in the area of the largest and smallest tissue section we found no statistical significant ($p = 0.4951$) difference in the area of the tumor and non-tumor group (Supplementary Fig. 2). Depending on the thicknesses (or number) of the OCT embedded sections the nanosensor reaction mix would contain varying concentration of OCT. A section of 5 μm consisting only of OCT compound (thus without tissue present) dissolved into 60 μL reaction mix would correspond to approximately 1% OCT compound, whereas 3 sections of 10 μm would correspond to approximately 6%. Therefore, the TDP1 and TOP1 activities were measured in the presence of increasing concentration of OCT compound up to 10% to examine if the OCT compound influenced the enzymatic measurements (Fig. 2C and D). OCT did not influence the TDP1 measurements at low concentration (0.625% to 2.5%), however, a minor reduction in the activity of TDP1 may be seen at 5% and a clear inhibition could be seen at 10% (Fig. 2C). For the TOP1 measurements no change in activity could be seen with varying concentration of OCT (Fig. 2D). However, in the NSCLC samples, the tissue on average occupied half the volume of the tube used for OCT embedding thus, 2 × 10 μm sections corresponded to a final concentration of approximately 2% OCT in the nanosensor reaction mix. The rest of the experiments were performed using tissue extract from 2 × 10 μm sections corresponding to approximately 2% OCT compound which did not influence the nanosensor measurements (Fig. 2C and D). Furthermore, this amount of tissue resulted in measurements well above the sample without tissue and should therefore enable TDP1 and TOP1 measurements even in samples with low enzyme activity (Fig. 2A and B).

3.2. TDP1 activity in NSCLC

It has previously been shown by Liu C et al. that in NSCLC TDP1 activity is upregulated in the tumor tissue compared to the adjacent non-tumor tissue (Liu et al., 2007). Their study only included 4 paired NSCLC tissue samples. However, they did also observe an upregulation of TDP1 activity in 9 NSCLC tissues compared to 3 non-neoplastic lung tissues. To examine if the activity of TDP1 was significantly upregulated in the tumor tissue in NSCLC compared to adjacent non-tumor samples, we measured TDP1 activity in cryosections from the paired NSCLC tissues using our previously published TDP1 nanosensor (Jensen et al., 2013).

Despite the relative small number of patient samples tested, we found that TDP1 was significantly ($p = 0.0001$) upregulated in the tumor samples compared to the non-tumor samples (Fig. 3A). Furthermore, due to the inclusion of both a tumor and an adjacent non-tumor sample from each patient a comparison between the tumor and non-tumor sample was possible. Interestingly, the activity of TDP1 was found to be upregulated in all but two tumor samples (20/22) compared to the paired non-tumor sample (Fig. 3B). These two “tumor” samples showing no increase in TDP1 activity were, however, subsequently

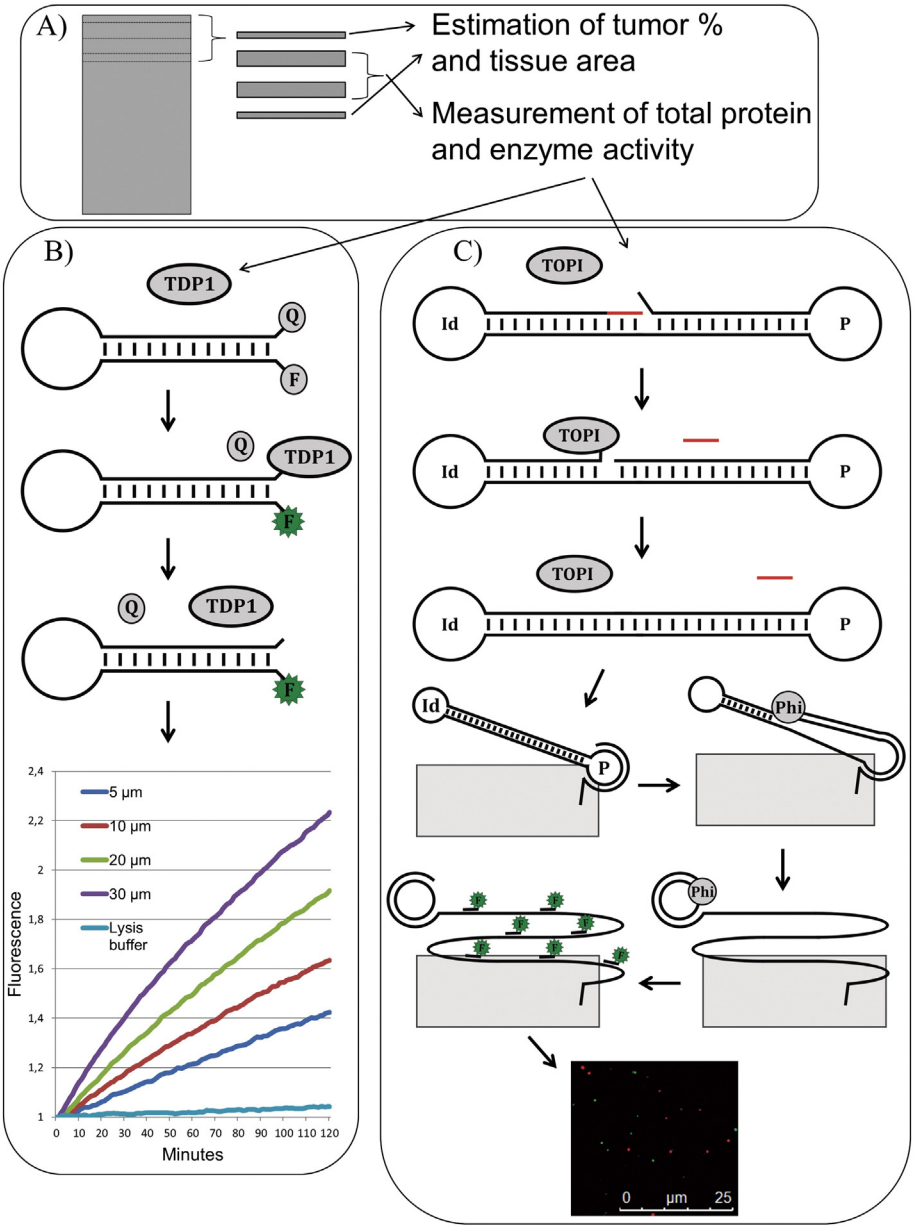

Fig. 1. Illustration of how the TDP1 and TOP1 measurements in cryosections are performed. A) The column in the upper left corner represents the OCT embedded tissue, which is cut into thin sections where the first and last are HE-stained and used for determining the area and the tumor percentage. The sections in between are dissolved in 1× TDP1 buffer, divided into three aliquots, and used for determining the protein concentration (by photospectrometric measurement), for measuring the activity of TDP1 (using the TDP1 nanosensor), and TOP1 (using the TOP1 nanosensor). B) The TDP1 nanosensor is composed of a DNA oligonucleotide labeled with an ATTO488 fluorophore in the 5′-end (marked with an F) and a BHQ1 quencher in the 3′-end (marked with a Q). ATTO488 and BHQ1 are brought into proximity through intramolecular folding of the DNA into a beacon like structure enabling BHQ1 to quench ATTO488. Upon addition of the TDP1 nanosensor to the tissue extract, the endogenous TDP1 removes BHQ1 from the nanosensor allowing detection of the emitted fluorescence from ATTO488. The increase in fluorescence over time corresponding to the TDP1 activity is subsequently measured using a real-time PCR machine. C) The TOP1 nanosensor, is composed of a DNA oligonucleotide designed to adopt a dumbbell structure consisting of a stretch of double-stranded DNA connected by two loops holding a sequence identifying the substrate (marked Id) and a primer binding sequence (marked P). Upon addition of the TOP1 nanosensor to the tissue extract, the endogenous TOP1 cleaves the nanosensor releasing three bases of DNA (shown in red) from the 3′end. This enables the 5′end to hybridize, and TOP1 can subsequently ligate the nanosensor into a closed circle. The generated circle is hybridized to a surface-attached primer matching the primer binding sequence. Subsequently, Phi29 DNA polymerase mix is added to support RCA and the resulting RCA products are visualized by hybridization of fluorescently labeled probes (matching the Id sequence) and the products analyzed using a fluorescence microscope. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found not to contain tumor cells (see below). To test if the increase in TDP1 activity correlated with the tumor percentage an experienced pathologist, without pre-knowledge of the results of the TDP1 activity measurements, estimated the tumor percentage in the two HE stained sections i.e. before and after the sections used for activity measurement (Fig. 1). Despite that the tumor percentage due to a lack of useable tumor markers in NSCLC was an estimation in steps of 10% based on cellular morphology rather than a precise count and that only 20 tumor samples were used for the correlation (tumor samples without any tumor cells, or with lacking data were excluded), we found a positive correlation ($p = 0.0493$) between the tumor percentage and the TDP1 activity (Fig. 3C).

3.3. TOP1 activity in NSCLC

The activity of TOP1 has been reported to be upregulated in the tumor tissue compared to the adjacent non-tumor tissue in several different cancer types e.g. colorectal, prostate, and kidney cancer (Guichard et al.,

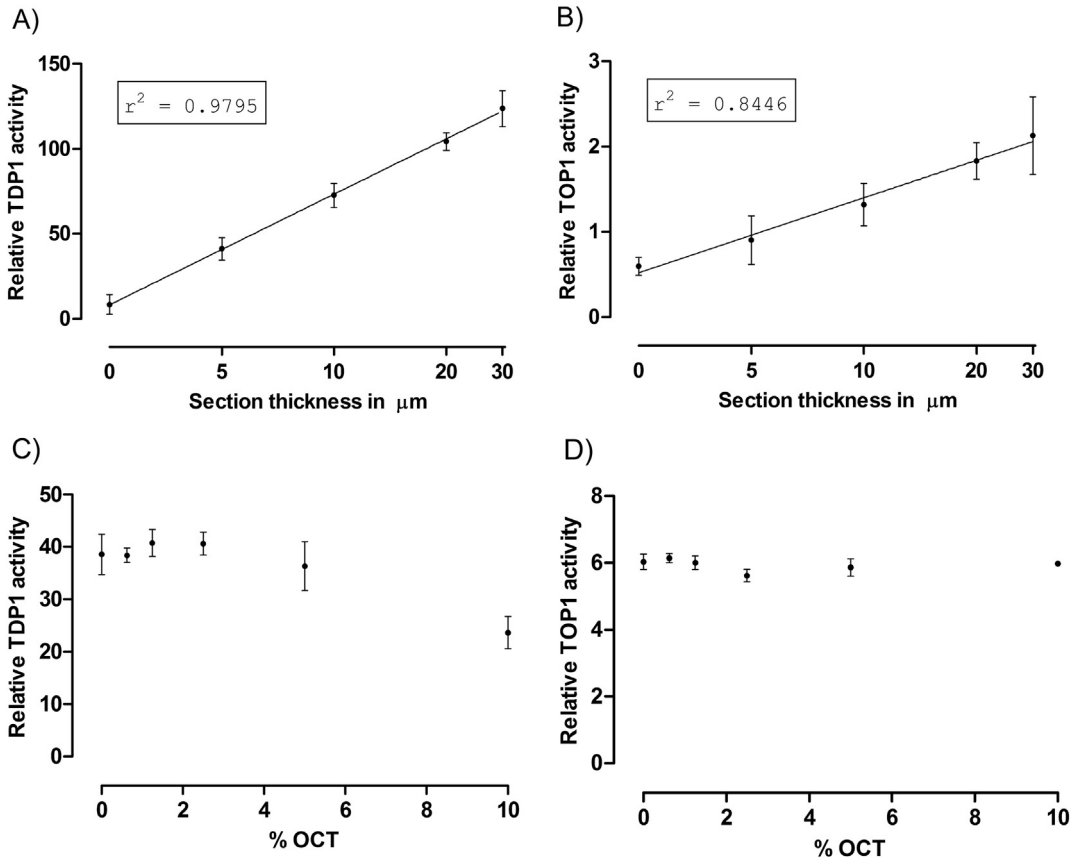

Fig. 2. Sensitivity of the TDP1 and TOP1 nanosensors in cryosections. A) TDP1 activity measured in varying thickness of cryosections (1 × 5 μm, 1 × 10 μm, 2 × 10 μm, and 3 × 10 μm) and depicted as mean from triplicate reactions ± SD in a XY plot. B) TOP1 activity in varying thickness of cryosections (1 × 5 μm, 1 × 10 μm, 2 × 10 μm, and 3 × 10 μm) and depicted as mean from triplicate reactions ± SD in a XY plot. C) TDP1 activity measured in the presence of varying concentration of OCT (0%, 0.625%, 1.25%, 2.5%, 5%, and 10%) and depicted as mean from triplicate reactions ± SD in a XY plot. D) TOP1 activity measured in the presence of varying concentration of OCT (0%, 0.625%, 1.25%, 2.5%, 5%, and 10%) and depicted as mean from triplicate reactions ± SD in a XY plot.

1999; Husain et al., 1994). Since a comparison of the TOP1 activity in the tumor and adjacent non-tumor tissue has not been performed in NSCLC we measured TOP1 activity in the cryosection extracts also used for TDP1 measurement using our previously published TOP1 nanosensor (Stougaard et al., 2009). We found that there was a significantly ($p = 0.0038$) higher activity of TOP1 in the tumor samples compared to the non-tumor samples (Fig. 4A). Furthermore, TOP1 was found to be upregulated in the tumor sample compared to the adjacent non-tumor sample from the same patient in approximately 78% (14/18) of the paired samples (Fig. 4B). When comparing the activity to the estimated tumor percentage we found four samples to have a decrease in TOP1 activity. The four samples had no, 30%, 60%, and 90% tumor cells. Lastly, we examined if the TOP1 activity correlated with the tumor percentage, but found no significant ($p = 0.8317$) correlation (Fig. 4C).

3.4. Correlation between the activities of TDP1 and TOP1 in NSCLC

Since the activity of both TDP1 and TOP1 was upregulated in the tumor samples compared to the non-tumor samples in NSCLC and the fact that TDP1 initially was discovered by its ability to remove TOP1 covalently trapped to the DNA we speculated if there could be a correlation between the two enzyme activities. Thus, we compared the activity of TDP1 and TOP1 and found a positive correlation both in the tumor samples ($p = 0.028$, Fig. 5A) and in the non-tumor samples ($p = 0.0075$, Fig. 5B). Furthermore, we examined if a correlation existed between the tumor specific change in the activities of TDP1 and TOP1, i.e. the relative change in activity from the non-tumor to the tumor sample for each patient. This was done by dividing the TDP1 and TOP1 activity measured in the tumor sample with the activity measured in corresponding non-tumor sample of the individual patients. Interestingly, there was a clear positive correlation ($p = 0.0037$) between the change in TDP1 activity and change in TOP1 activity (Fig. 5C).

4. Discussion

In the present study we examined the activities of TOP1 and TDP1 in cryosections from NSCLC tissue samples. Most of the knowledge about enzyme activities such as TOP1 and TDP1 comes from studies based on cell lines. Although cell line based studies are invaluable due to the possibilities for modifying activities through transfection or knockdown, they are very different from tissue samples and thus may not reflect a true picture of the enzymatic activities in tumors. Traditionally, most studies using tissue sections have been based on detecting proteins (Immunohistochemistry) or DNA/RNA (in situ hybridization or PCR) rather than the enzymatic activities. Although these techniques are very useful for identifying intra- and inter-cellular expression or for identifying mutational status they say little about the biologic function of the enzymes since posttranslational modifications may alter the biological important enzymatic activities. Both TDP1 and TOP1 have been reported to be posttranslational modified (Bandyopadhyay and Gjerset, 2011; Chiang et al., 2010; Das et al., 2009; Hudson et al., 2012). But whereas both TOP1 activity and drug interaction may be altered by posttranslational modifications (Roy et al., 2014), no posttranslational modifications of TDP1 yet have been reported to influence the catalytic activity. In a recent study by Meisenberg et al. on six colorectal cancer cell lines a correlation between TDP1 protein and TDP1 activity was detected (Meisenberg et al., 2015). However, Liu C et al. have previously reported that TDP1 protein does not necessarily correlate with

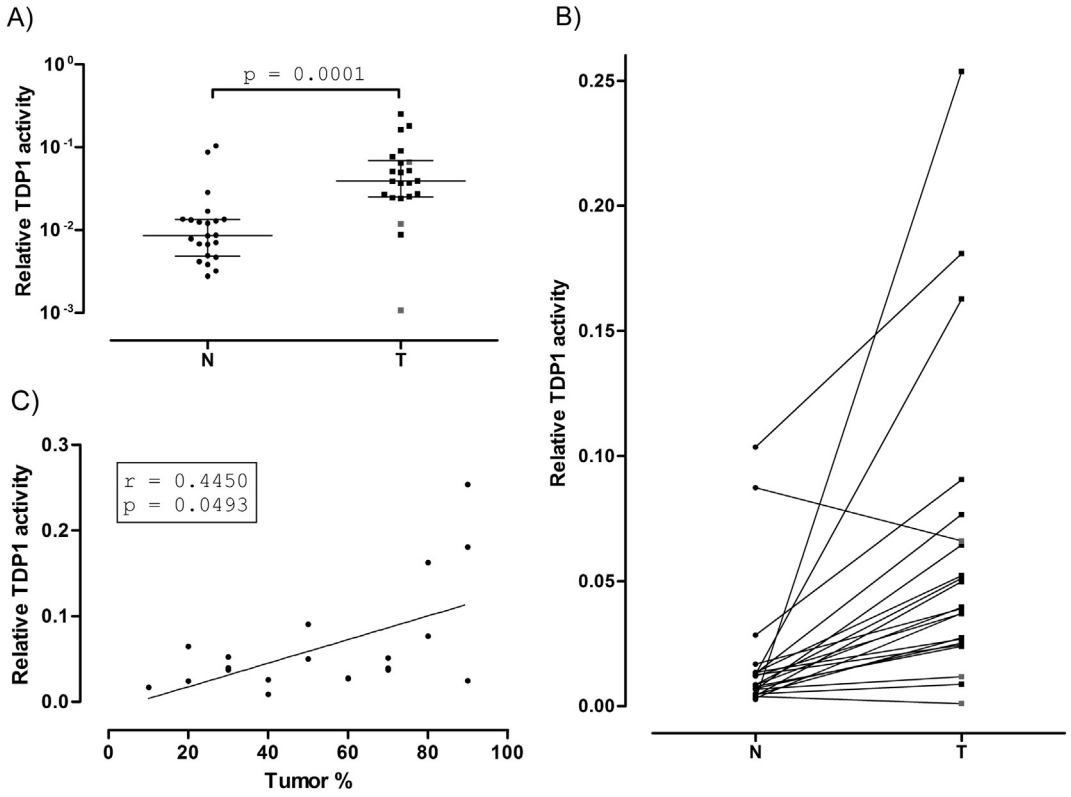

Fig. 3. Measurement of TDP1 activity in tumor and non-tumor samples from NSCLC patients. Two sections of 10 μm thickness were used from each sample. The samples marked in gray in the tumor group were found not to contain any tumor cells in the surrounding sections (the first and last section, see Fig. 2). A) Comparison of the measured TDP1 activity in the tumor and the non-tumor samples, depicted as the median with interquartile range in a scatter dot plot. B) Graphical representation of the measured TDP1 activity where the samples from each patient, tumor (T) and non-tumor (N), were connected by a line indicating whether the TDP1 activity in each patient is increased in the tumor compared to the non-tumor sample. C) Correlation of the measured TDP1 activity in the tumor samples to the tumor percentage.

TDP1 activity in tissue extract from NSCLC (Liu et al., 2007) suggesting that TDP1 activity could be posttranslationally regulated. The contradicting observations in the two studies could be due to the different origins (colon cancer vs. lung cancer) or due to the different sample types (cell lines vs. tissue samples).

The activity measurements were performed using extract from cryosections rather than whole pieces of tissue since this allowed morphological determination of the tumor percentage and tissue area. Here we demonstrated that the sensitivity of the nanosensors used allows precise measurement of both TOP1 and TDP1 activities in cryosections down to 5 μm thickness (Fig. 2A and B). However, we would suggest to use thicker sections (we used 2 × 10 μm) since this will maximize the chance of getting precise activity measurements in samples with low TOP1 and TDP1 activities and the final concentration of OCT compound should be kept below 5% to avoid it from influencing the measurements (Fig. 2C and D).

We found the activity of TDP1 to be 3.5-fold upregulated ($p = 0.0001$) in the tumor samples compared to the non-tumor samples (Fig. 3). This is in accordance with the previous findings by Liu C et al. who found TDP1 to be upregulated in 9 non-paired NSCLC tissues compared to 3 non-neoplastic lung tissues and in 4 paired NSCLC tissues (Liu et al., 2007). Interestingly when we compared the TDP1 activity in the individual tumor samples to the adjacent non-tumor sample (thus the tumor sample and non-tumor sample from the same patient) all but two had higher activity in the tumor sample. This could, however, be explained by a lack of tumor cells in these two samples. The finding of all tumor samples having increased TDP1 activity compared to the non-tumor sample of the same patient indicates that upregulation of TDP1 activity must be highly beneficial for the tumor cells. This may be a consequence of the tumor cells becoming more dependent on the TDP1-PARP1 dependent pathway due to loss of complementary repair pathways e.g. checkpoint dependent DNA repair pathways.

TDP1 has long been suggested to be an interesting chemotherapeutic target (Beretta et al., 2010; Dexheimer et al., 2008; Hirano et al., 2007; Pommier et al., 2014) and may be a particularly interesting drug target in NSCLC since all tumor samples had increased TDP1 activity. Furthermore, despite the limited amount of samples we found a positive correlation ($p = 0.0493$) of TDP1 activity to the tumor percentage. Liu C et al. used extract from 50–100 ng snap frozen tissue ground in a mortar thus the tumor percentage was not reported although it was stated that the tumor tissue was microdissected to greater than 60% purity. In our experience it can however be difficult to know if indeed tumor samples are present in a sample unless sections can be evaluated microscopically, which is why it is preferable to determine the tumor percentage as an average of the sections from either side of the tissue used for activity measurement (Fig. 1).

The activity of TOP1 has not previously been compared between tumor and non-tumor tissues in NSCLC. Though, it has been compared between different types of cancers including lung cancer (McLeod et al., 1994) where a large variation in TOP1 activity both within and between different types of cancers was reported. However, the study did not include any non-cancerous lung tissue. Similar to this finding we also saw a large variation in the activity of TOP1 in tumor samples from different patients (Fig. 4) and this difference did not correlate ($p = 0.8317$) with the tumor percentage (Fig. 4C). Comparing the activity of TOP1 between the tumor and non-tumor samples we found TOP1 to be 2.9-fold upregulated ($p = 0.0038$) in the tumor samples compared to the non-tumor samples (Fig. 4A) as also reported in several other types of cancer (Guichard et al., 1999; Husain et al., 1994; Masin et al., 1995). When comparing the TOP1 activity in individual tumor samples to the non-tumor samples we found TOP1 to be upregulated in 78% (14/

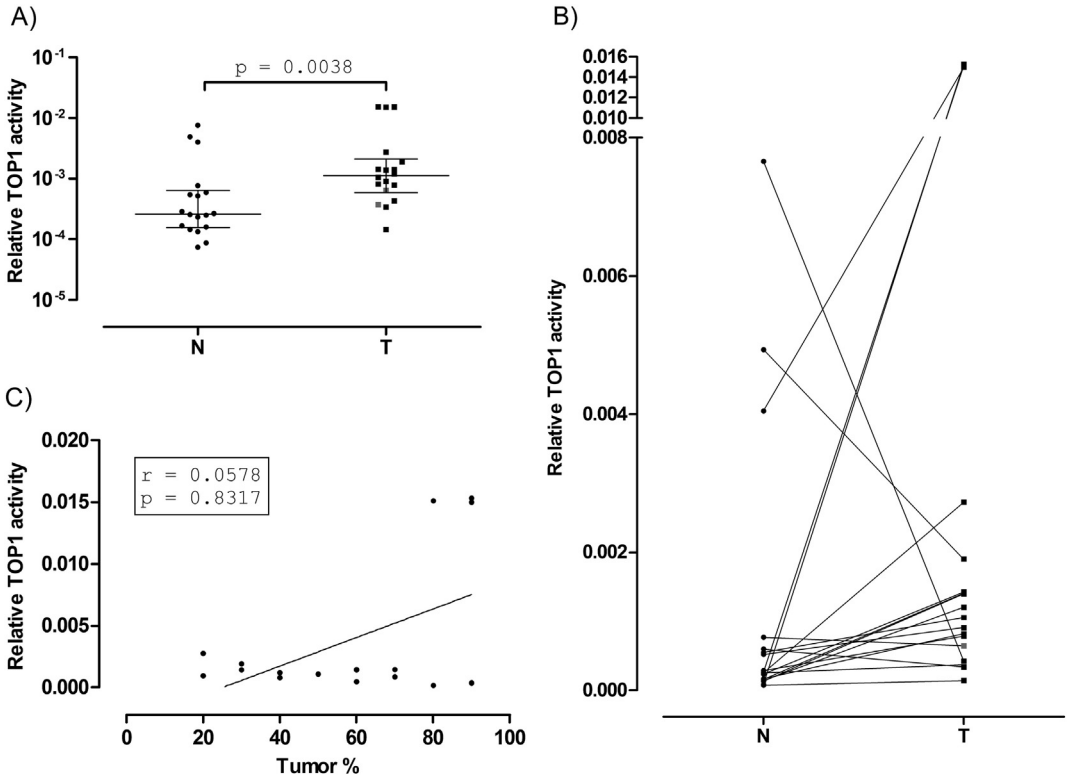

Fig. 4. Measurement of TOP1 activity in tumor and non-tumor samples from NSCLC patients. Two sections of 10 μm thickness were used from each sample. The samples marked in gray in the tumor group were found not to contain any tumor cells in the surrounding sections (the first and last section, see Fig. 2). A) Comparison of the measured TOP1 activity in the tumor and the non-tumor samples, depicted as the median with interquartile range in a scatter dot plot. B) Graphical representation of the measured TOP1 activity where the samples from each patient, tumor (T) and non-tumor (N), were connected by a line indicating whether the TOP1 activity in each patient is increased in the tumor compared to the non-tumor sample. C) Correlation of the measured TOP1 activity in the tumor samples to the tumor percentage.

18) and 82% (14/17) if disregarding the sample without tumor cells (Fig. 4B).

Since drugs of the CPT family such as irinotecan and topotecan have been proposed as treatments in NSCLC (Chen et al., 2012; O'Brien et al., 2007; Tsakalozou et al., 2014) and exhibit cytotoxicity by converting the endogenous TOP1 activity into a cell poison, the level of TOP1 activity present in the tumor cells is a major factor in predicting a response. However, both downregulation of TOP1 activity (Burgess et al., 2008; Sorensen et al., 1995) and high TDP1 activity have been reported to counteract CPT induced DNA damage (Barthelmes et al., 2004; Nivens et al., 2004). TOP1-DNA complexes may be stabilized not only by CPT but also by natural occurring DNA lesions e.g. caused by UV irradiation, oxidative DNA damage, telomeric shortening, or replication stress induced by oncogene activation (Bartek et al., 2007; Daroui et al., 2004; Luo et al., 2009). From a biological point of view it would make sense that increased TOP1 activity would require increased DNA repair capability. Although different repair pathways are involved in the repair of TOP1 induced DNA damage, the importance of TDP1 is illustrated by the fact that cells from SCAN1 patients homozygote for the A1478G mutation and TDP1 deficient mouse cells are hypersensitive to CPT (Hirano et al., 2007; Interthal et al., 2005b; Miao et al., 2006). Furthermore, TDP1 depletion in colorectal cell lines has been demonstrated to increase the sensitivity to irinotecan treatment (Meisenberg et al., 2015). Due to the functionally linked activities, we speculated if there could be a positive correlation between TOP1 activity and TDP1 activity in the tested tissue samples. Despite the limited number of samples included we found a clear positive correlation between the TOP1 and TDP1 activities both in the tumor ($p = 0.028$) and in the non-tumor ($p = 0.0075$) samples (Fig. 5A and B). Furthermore, this correlation became even more obvious ($p = 0.0037$) when comparing the change in TDP1 activity and change in TOP1 activity thus the difference in activity in the tumor and non-tumor samples from the individual patients. What causes this correlation is unknown, however, it is intriguing to speculate if it is a consequence of a controlled feedback mechanism either directly where high TOP1 activity causes increased TDP1 activity or indirectly if TOP1 and TDP1 shares common regulators of expression or activity. Either way it suggests that there could be a benefit of combining CPT based therapy with drugs targeting TDP1. Dual inhibitors targeting both TDP1 and TOP1 have been reported (Conda-Sheridan et al., 2013) but these are not yet approved for use in patients. Since there are no TDP1 inhibitors currently approved for use in patients a possibility could be to combine CPT with PARP inhibitors, as previously suggested (Gilbert et al., 2012; Znojek et al., 2014). This has been tried with success in childhood neuroblastoma (Daniel et al., 2009), however, a phase 1 study in refractory solid tumors and lymphomas examining combined treatment with CPT and PARP inhibitors was terminated before time due to toxicity (Kummar et al., 2011).

In summary, we have used our previously published nanosensors to measure the enzymatic activity of TOP1 and TDP1 in cryosections from NSCLC patients. We found a significant upregulation of both TOP1 and TDP1 activities in the tumor compared to the non-tumor tissue of the NSCLC patients ($p = 0.0038$ and $p = 0.0001$, respectively). TOP1 was upregulated in 82% (14/17) with no correlation to the tumor percentage ($p = 0.8317$), whereas TDP1 was upregulated in 100% (20/20) with a positive correlation to the tumor percentage ($p = 0.0493$). We found a positive correlation between the TOP1 and TDP1 activities both in the tumor ($p = 0.028$) and in the non-tumor ($p = 0.0075$) samples, and this correlation became even more pronounced when comparing the change in activities for the individual patients ($p = 0.0037$). Although the results here presented are based on a small cohort and should be verified in a larger cohort, they illustrate that TDP1 and TOP1 activity measurements can be used in non-fixed tissue biopsies such as cryosections. Furthermore, the correlation between TOP1 and TDP1 activities suggests that it could be advantageous to combine CPT

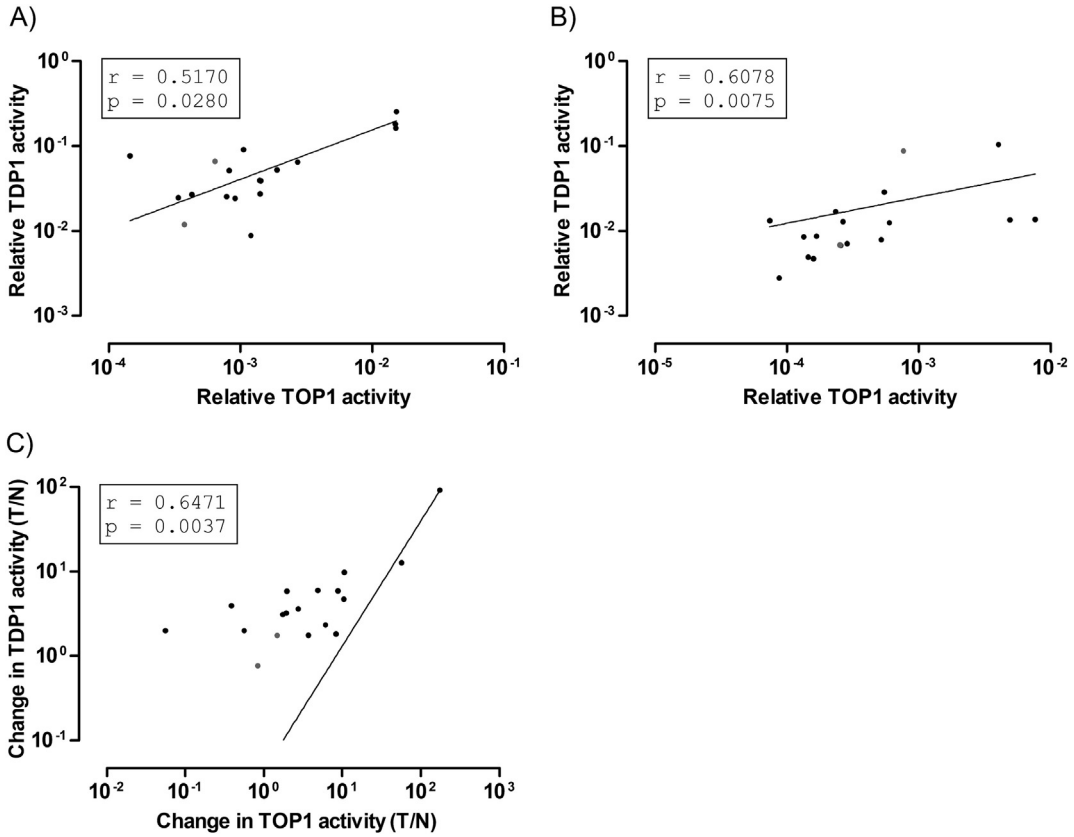

Fig. 5. Correlation of the measured TDP1 and TOP1 activities in tumor and non-tumor samples from NSCLC patients. A) Correlation of the measured TDP1 and TOP1 activities in the tumor samples. B) Correlation of the measured TDP1 and TOP1 activities in the normal samples. C) Correlation of the change in activity (thus T/N) of TDP1 and TOP1.

based therapy with TDP1 inhibitors in NSCLC. Since a correlation between the activities of TOP1 and TDP1 was found both in the tumor and the non-tumor group, it is tempting to speculate that it could be the result of co-regulation of the activities and thus present in other types of tissues and cancers, however, this remains to be examined.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yexmp.2015.05.006.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by grants from the Aase og Ejnar Danielsens Fond, the Købmand Svend Hansen og hustru Ina Hansens Fond, the Familien Hede Nielsens Fond, the Marie & M. B. Richters Fond, the Fabrikant Einar Willumsens Mindelegat, the Kong Christian Den Tiendes Fond, the Lykfeldts legat, the Fhv. Dir. Leo Nielsen og Hustru Karen Margrethe Nielsens Legat for Lægevidenskabelig Grundforskning, and the Aage og Johanne Luis-Hansens Fond. We thank Noriko Hansen for technical assistance during this project.

References

Bandyopadhyay, K., Gjerset, R.A., 2011. Protein kinase CK2 is a central regulator of topoisomerase I hyperphosphorylation and camptothecin sensitivity in cancer cell lines. Biochemistry 50, 704–714.
Bartek, J., et al., 2007. DNA damage signalling guards against activated oncogenes and tumour progression. Oncogene 26, 7773–7779.
Barthelmes, H.U., et al., 2004. TDP1 overexpression in human cells counteracts DNA damage mediated by topoisomerases I and II. J. Biol. Chem. 279, 55618–55625.
Ben Hassine, S., Arcangioli, B., 2009. Tdp1 protects against oxidative DNA damage in non-dividing fission yeast. EMBO J. 28, 632–640.
Beretta, G.L., et al., 2010. Tyrosyl-DNA phosphodiesterase 1 targeting for modulation of camptothecin-based treatment. Curr. Med. Chem. 17, 1500–1508.
Burgess, D.J., et al., 2008. Topoisomerase levels determine chemotherapy response in vitro and in vivo. Proc. Natl. Acad. Sci. U. S. A. 105, 9053–9058.
Champoux, J.J., 2001. DNA topoisomerases: structure, function, and mechanism. Annu. Rev. Biochem. 70, 369–413.
Chen, A.Y., et al., 2012. DNA topoisomerase I drugs and radiotherapy for lung cancer. J. Thorac. Dis. 4, 390–397.
Chiang, S.C., et al., 2010. TDP1 serine 81 promotes interaction with DNA ligase IIIalpha and facilitates cell survival following DNA damage. Cell Cycle 9, 588–595.
Conda-Sheridan, M., et al., 2013. Synthesis and biological evaluation of indenoisoquinolines that inhibit both tyrosyl-DNA phosphodiesterase I (Tdp1) and topoisomerase I (Top1). J. Med. Chem. 56, 182–200.
Daniel, R.A., et al., 2009. Inhibition of poly(ADP-ribose) polymerase-1 enhances temozolomide and topotecan activity against childhood neuroblastoma. Clin. Cancer Res. 15, 1241–1249.
Daroui, P., et al., 2004. Hydrogen peroxide induces topoisomerase I-mediated DNA damage and cell death. J. Biol. Chem. 279, 14587–14594.
D'Arpa, P., et al., 1990. Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res. 50, 6919–6924.
Das, B.B., et al., 2009. Optimal function of the DNA repair enzyme TDP1 requires its phosphorylation by ATM and/or DNA-PK. EMBO J. 28, 3667–3680.
Das, B.B., et al., 2010. Role of tyrosyl-DNA phosphodiesterase (TDP1) in mitochondria. Proc. Natl. Acad. Sci. U. S. A. 107, 19790–19795.
Das, B.B., et al., 2014. PARP1-TDP1 coupling for the repair of topoisomerase I-induced DNA damage. Nucleic Acids Res. 42, 4435–4449.
Dexheimer, T.S., et al., 2008. Tyrosyl-DNA phosphodiesterase as a target for anticancer therapy. Anticancer Agents Med. Chem. 8, 381–389.
Do, H., et al., 2014. A critical re-assessment of DNA repair gene promoter methylation in non-small cell lung carcinoma. Sci. Rep. 4, 4186.
El-Khamisy, S.F., et al., 2005. Defective DNA single-strand break repair in spinocerebellar ataxia with axonal neuropathy-1. Nature 434, 108–113.
Eng, W.K., et al., 1988. Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. Mol. Pharmacol. 34, 755–760.
Fam, H.K., et al., 2013. Expression profile and mitochondrial colocalization of Tdp1 in peripheral human tissues. J. Mol. Histol. 44, 481–494.
Fruh, M., et al., 2013. Small-cell lung cancer (SCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 24 (Suppl. 6), vi99–vi105.
Giaccone, G., et al., 1995. Differential expression of DNA topoisomerases in non-small cell lung cancer and normal lung. Biochim. Biophys. Acta 1264, 337–346.
Gilbert, D.C., et al., 2012. Topoisomerase I inhibition in colorectal cancer: biomarkers and therapeutic targets. Br. J. Cancer 106, 18–24.

Goldwasser, F., et al., 1995. Topoisomerase I-related parameters and camptothecin activity in the colon carcinoma cell lines from the National Cancer Institute anticancer screen. Cancer Res. 55, 2116–2121.
Guichard, S., et al., 1999. CPT-11 converting carboxylesterase and topoisomerase activities in tumour and normal colon and liver tissues. Br. J. Cancer 80, 364–370.
Hartwell, D., et al., 2011. Topotecan for relapsed small cell lung cancer: a systematic review and economic evaluation. Cancer Treat. Rev. 37, 242–249.
Hirano, R., et al., 2007. Spinocerebellar ataxia with axonal neuropathy: consequence of a Tdp1 recessive neomorphic mutation? EMBO J. 26, 4732–4743.
Huang, S.Y., et al., 2013. TDP1 repairs nuclear and mitochondrial DNA damage induced by chain-terminating anticancer and antiviral nucleoside analogs. Nucleic Acids Res. 41, 7793–7803.
Hudson, J.J., et al., 2012. SUMO modification of the neuroprotective protein TDP1 facilitates chromosomal single-strand break repair. Nat. Commun. 3, 733.
Husain, I., et al., 1994. Elevation of topoisomerase I messenger RNA, protein, and catalytic activity in human tumors: demonstration of tumor-type specificity and implications for cancer chemotherapy. Cancer Res. 54, 539–546.
Inamdar, K.V., et al., 2002. Conversion of phosphoglycolate to phosphate termini on 3′ overhangs of DNA double strand breaks by the human tyrosyl-DNA phosphodiesterase hTdp1. J. Biol. Chem. 277, 27162–27168.
Interthal, H., et al., 2005a. Human Tdp1 cleaves a broad spectrum of substrates, including phosphoamide linkages. J. Biol. Chem. 280, 36518–36528.
Interthal, H., et al., 2005b. SCAN1 mutant Tdp1 accumulates the enzyme–DNA intermediate and causes camptothecin hypersensitivity. EMBO J. 24, 2224–2233.
Jansen, W.J., et al., 1997. CPT-11 in human colon-cancer cell lines and xenografts: characterization of cellular sensitivity determinants. Int. J. Cancer 70, 335–340.
Jensen, P.W., et al., 2013. Real-time detection of TDP1 activity using a fluorophore-quencher coupled DNA-biosensor. Biosens. Bioelectron. 48, 230–237.
Kalemkerian, G.P., et al., 2013. Small cell lung cancer. J. Natl. Compr. Canc. Netw. 11, 78–98.
Kummar, S., et al., 2011. Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas. Cancer Res. 71, 5626–5634.
Liu, C., et al., 2002. Repair of topoisomerase I covalent complexes in the absence of the tyrosyl-DNA phosphodiesterase Tdp1. Proc. Natl. Acad. Sci. U. S. A. 99, 14970–14975.
Liu, C., et al., 2007. Increased expression and activity of repair genes TDP1 and XPF in non-small cell lung cancer. Lung Cancer 55, 303–311.
Luo, J., et al., 2009. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 136, 823–837.
Masin, J.S., et al., 1995. Topoisomerase I activity in squamous cell carcinoma of the head and neck. Laryngoscope 105, 1191–1196.
McLeod, H.L., et al., 1994. Topoisomerase I and II activity in human breast, cervix, lung and colon cancer. Int. J. Cancer 59, 607–611.
Meisenberg, C., et al., 2015. Clinical and cellular roles for TDP1 and TOP1 in modulating colorectal cancer response to irinotecan. Mol. Cancer Ther. 14, 575–585.
Miao, Z.H., et al., 2006. Hereditary ataxia SCAN1 cells are defective for the repair of transcription-dependent topoisomerase I cleavage complexes. DNA Repair (Amst) 5, 1489–1494.
Naumann, R.W., Coleman, R.L., 2011. Management strategies for recurrent platinum-resistant ovarian cancer. Drugs 71, 1397–1412.
Nivens, M.C., et al., 2004. Engineered resistance to camptothecin and antifolates by retroviral coexpression of tyrosyl DNA phosphodiesterase-I and thymidylate synthase. Cancer Chemother. Pharmacol. 53, 107–115.
O'Brien, M., et al., 2007. Recent advances with topotecan in the treatment of lung cancer. Oncologist 12, 1194–1204.
Plo, I., et al., 2003. Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. DNA Repair (Amst) 2, 1087–1100.
Pommier, Y., 2006. Topoisomerase I inhibitors: camptothecins and beyond. Nat. Rev. Cancer 6, 789–802.
Pommier, Y., et al., 2014. Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). DNA Repair (Amst) 19, 114–129.
Pourquier, P., et al., 1997a. Trapping of mammalian topoisomerase I and recombinations induced by damaged DNA containing nicks or gaps. Importance of DNA end phosphorylation and camptothecin effects. J. Biol. Chem. 272, 26441–26447.
Pourquier, P., et al., 1997b. Effects of uracil incorporation, DNA mismatches, and abasic sites on cleavage and religation activities of mammalian topoisomerase I. J. Biol. Chem. 272, 7792–7796.
Proszek, J., et al., 2013. Topoisomerase I as a biomarker: detection of activity at the single molecule level. Sensors (Basel) 14, 1195–1207.
Roberts, P.J., Stinchcombe, T.E., 2013. KRAS mutation: should we test for it, and does it matter? J. Clin. Oncol. 31, 1112–1121.
Roy, A., et al., 2014. Decreased camptothecin sensitivity of the stem-cell-like fraction of Caco2 cells correlates with an altered phosphorylation pattern of topoisomerase I. PLoS One 9, e99628.
Sorensen, M., et al., 1995. Characterisation of a human small-cell lung cancer cell line resistant to the DNA topoisomerase I-directed drug topotecan. Br. J. Cancer 72, 399–404.
Stein, A., Arnold, D., 2012. Oxaliplatin: a review of approved uses. Expert. Opin. Pharmacother. 13, 125–137.
Steuer, C.E., Ramalingam, S.S., 2014. ALK-positive non-small cell lung cancer: mechanisms of resistance and emerging treatment options. Cancer 120, 2392–2402.
Stougaard, M., Ho, Y.P., 2013. DNA-based nanosensors for next-generation clinical diagnostics via detection of enzyme activity. Expert. Rev. Mol. Diagn. 14, 1–3.
Stougaard, M., et al., 2009. Single-molecule detection of human topoisomerase I cleavage-ligation activity. ACS Nano 3, 223–233.
Takashima, H., et al., 2002. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. Nat. Genet. 32, 267–272.
Tsakalozou, E., et al., 2014. Protracted dosing of the lipophilic camptothecin analogue AR-67 in non-small cell lung cancer xenografts and humans. Cancer Chemother. Pharmacol. 74, 45–54.
Yang, S.W., et al., 1996. A eukaryotic enzyme that can disjoin dead-end covalent complexes between DNA and type I topoisomerases. Proc. Natl. Acad. Sci. U. S. A. 93, 11534–11539.
Zhang, Y.W., et al., 2011. Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells. Nucleic Acids Res. 39, 3607–3620.
Zhou, T., et al., 2005. Deficiency in 3′-phosphoglycolate processing in human cells with a hereditary mutation in tyrosyl-DNA phosphodiesterase (TDP1). Nucleic Acids Res. 33, 289–297.
Znojek, P., et al., 2014. Preferential potentiation of topoisomerase I poison cytotoxicity by PARP inhibition in S phase. Br. J. Cancer 111, 1319–1326.