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

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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

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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