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Epigenetic and genetic inactivation of tyrosyl-DNA-phosphodiesterase 1 (TDP1) in human lung cancer cells from the NCI-60 panel☆

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ABSTRACT

Tyrosyl-DNA-phosphodiesterase 1 (TDP1) repairs 3′-blocking DNA lesions by catalytically hydrolyzing the tyrosyl-DNA-phosphodiester bond of trapped topoisomerase I (Top1) cleavage complexes (Top1cc). It also removes 3′-blocking residues derived from oxidative damage or incorporation of chain terminating anticancer and antiviral nucleosides. Thus, TDP1 is regarded as a determinant of resistance to Top1 inhibitors and chain terminating nucleosides, and possibly of genomic stability. In the 60 cell lines of the NCI Developmental Therapeutic Anticancer Screen (the NCI-60), whose whole genome transcriptome and mutations have recently been characterized, we discovered two human lung cancer cell lines deficient for TDP1 (NCI_H522 and HOP_62). HOP_62 shows undetectable *TDP1* mRNA and NCI_H522 bears a homozygous deleterious mutation of *TDP1* at a highly conserved amino acid residue (K292E). Absence of TDP1 protein and lack of TDP1 catalytic activity were demonstrated in cell lysates from both cell lines. Lack of TDP1 expression in HOP_62 was shown to be due to *TDP1* promoter hypermethylation. Our study provides insights into the possible inactivation of TDP1 in cancers and its relationship to cellular response to Top1-targeted drugs. It also reveals two TDP1 knockout lung cancer cell lines for further TDP1 functional analyses.

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1. Introduction

TDP1 is a nuclear and mitochondrial enzyme conserved in eukaryotes [1–3]. It hydrolyzes phosphodiester bonds between DNA 3′-phosphates and the active site tyrosine residue of topoisomerase I (Top1) [2,4,5] as well as a variety of other DNA 3′-blocking lesions resulting from oxidative damage (3′-phosphoglycolates) [3,6–8], alkylation damage [8] or incorporation of chain-terminating antiviral and anticancer nucleotide analogs [9]. TDP1 has also been implicated as a backup pathway for the repair of topoisomerase II cleavage complexes [8,10] and as regulating the fidelity of nonhomologous end joining [11]. The biological significance of TDP1 is also emphasized by the fact that a TDP1 mutation causes the human genetic disease, spinocerebellar ataxia with axonal neuropathy (SCAN1) [12].

DNA topoisomerases are crucial for regulating the topology of the genome and removing DNA supercoiling resulting form transcription, replication and chromatin dynamics [13,14]. They also are important targets for anti-cancer therapies. Top1 inhibitors represent widely used anticancer agents, and camptothecin (CPT) derivatives are prescribed for ovarian and small-cell lung cancers (topotecan) and for colorectal cancer (Irinotecan) [15]. These drugs act by trapping Top1-DNA complexes [14]. Top1-DNA complexes can also be trapped by a wide range of endogenous and exogenous DNA lesions [16]. Thus, the repair of Top1 cleavage complex by TDP1 is relevant for genomic stability and cancer therapy. TDP1 itself is a potential target for novel anti-cancer drugs, especially in the light of its relevance to tumor response to CPT derivatives. Efforts are ongoing to develop effective and safe inhibitors of TDP1 [17,18] to augment tumor sensitivity to Top1 inhibitors.

The NCI-60 panel of human cancer cell-lines is derived from nine different tissues of origin. It was initially developed for anticancer drug efficacy screening by the Developmental Therapeutics

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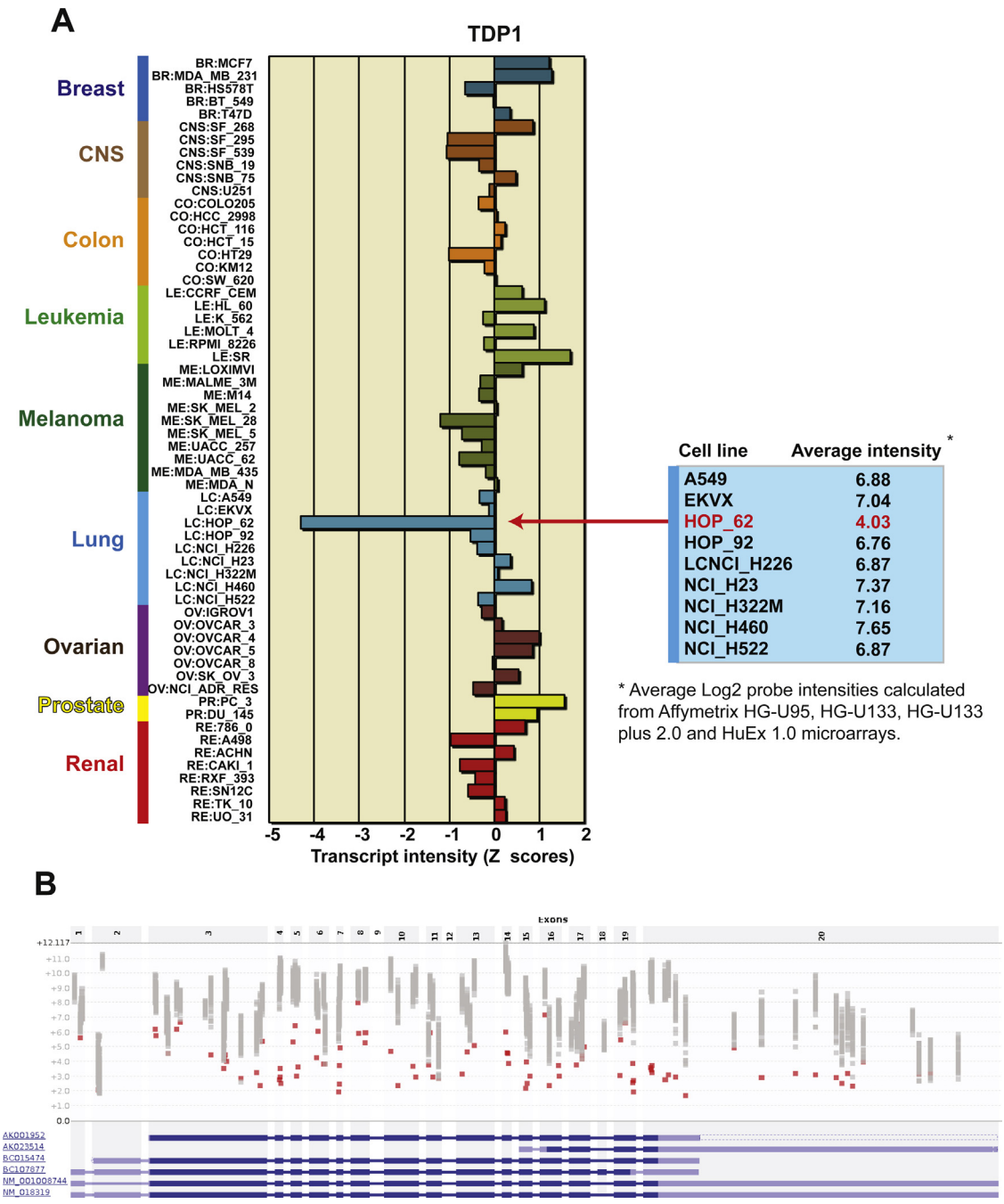

Fig. 1. TDP1 gene expression and mutations for the NCI-60 human tumor cell lines. (A) Relative TDP1 gene expression profile. Bars to the right show increased expression, bars to the left show decreased expression relative to the expression mean. Expression values are normalized as *z*-scores (described in [21]). The horizontal axis is marked in standard deviations from the mean. Right blue box: average probe intensity calculated from Affymetrix HG-U95, HG-U133, HG-U133 plus 2.0 and HuEx 1.0 microarrays. Data are accessible at http://discover.nci.nih.gov/cellminer. (B) TDP1 mRNA expression for all probe sets from the Affymetrix GeneChip Human Exon 1.0 ST (GH Exon 1.0 ST) for the NCI-60 cells (gray squares). Red squares with value at background level are for HOP_62. Reference gene structures are shown at the bottom as blue ribbons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Program (DTP) of the US National Cancer Institute. In addition, the NCI-60 has been extensively characterized in biological, molecular and pharmacological studies [19–22]. The complete NCI-60 genomic databases have recently been made publicly available including transcript levels across multiple platforms [21] and mutations of approximately 21,000 genes by whole human exome sequencing [22], enabling non-bioinformaticists to query the largest publicly available database of gene expression, mutations, microRNA, drugs and investigational compounds.

Here we examined the expression and genetic mutation profiles of *TDP1* in the NCI-60. With the availability of CellMiner (http://discover.nci.nih.gov/cellminer/), a web application for rapid retrieval of genetic and pharmacological data from the NCI-60 [21,22], this task is made feasible and relatively easy. We demonstrate how integration of bioinformatics and biological investigation lead to the identification of two TDP1-deficient cell lines from the NCI-60 cell panel.