**Materials and Methods**

*Cell Lines*

An MF-derived malignant T-cell line (MyLa2059) and ALCL-derived malignant T-cell lines (Mac-1, PB2B, Mac2A) were cultured in full media containing RPMI-1640, 100 mg/ml penicillin/streptomycin (P/S) and 10% fetal bovine serum (FBS) [28]. The Sézary syndrome (SS)- derived malignant T-cell lines SeAx and Sez4 were cultured in RPMI-1640 with 100 mg/ml P/S, 10% human serum (HS; Bloodbank, Copenhagen University Hospital, Copenhagen, Denmark) and U/ml IL-2. The MF-derived non-malignant T-cell line MyLa1850 was cultured in RPMI-1640 with 100 mg/ml P/S, 10% HS, U/ml IL-2 and 2.5 × U/ml IL-4.

*Protein Extraction and Western Blotting*

Protein extraction and Western blotting were performed as previously described [1, 2]. Protein concentration was measured by the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA).

As primary antibodies were used mouse anti-TXNIP (1:500; #K020503, MBL/Medical & Biological Laboratories), mouse anti-GAPDH (1:10,000) and mouse anti-GFP (1:1,000; Clontech, France). Secondary antibodies used were HRP-conjugated polyclonal rabbit anti-mouse Ig (1:2,000; DAKO).

*Quantitative PCR Analysis*

Total RNA was purified with an RNeasy Plus Mini Kit (Qiagen, Germany). cDNA was synthesized from 1.0 μg RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The quantification of gene expression was performed by using a Taqman Gene Expression assay (Applied Biosystems, USA) according to the manufacturer’s instructions. GAPDH was used as a reference gene. Amplification was performed in an MX3005P thermal cycler (Agilent Technologies, Santa Clara, CA, USA) on standard settings. Each experiment included 3 technical replicates.

*Bisulphite Sequencing*

250 ng of DNA was bisulphite treated to convert unmethylated cytosine to uracil using the EZ Methylation kit (Zymo Research) according to the manufacturer’s instructions. The DNA methylation status of the TXNIP promoter regions were analysed in CTCL cell lines and compared to whole blood from 11 healthy controls. Methylation-independent assays were designed using the PyroMark Assay Design 2.0 (Qiagen). The PCR was initiated with 15 min at 95°C for activating the DNA polymerase followed by 45 cycles of 30-s denaturation at 95°C, 30-s annealing and 30-s elongation at 72°C. Final elongation was carried out for 10 min at 72°C, and the PCR amplicons were held at 4°C until sequencing. The PCR amplicons were pyrosequenced on the PyroMark Q24 (Qiagen) instrument using the PyroMark Gold Q24 reagents (Qiagen) according to the manufacturers’ instructions. The DNA methylation level is calculated as the median DNA methylation level of the CpG sites included in the assay. Primer sequences are given in suppl. Table 1.

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| --- | --- | --- | --- | --- |
| Assay | Primer sequence | Number of CpG sites analysed | Annealing temperature, °C | Genomic location of the amplicon (hg38) |
| TXNIP F2:  TXNIP R\_bio\_3:  TXNIP seq\_2: | 5’-ggtTagtgggatTTtTTttTTaTtgga  5’-bio-ccattAActAccccAtccttAtttaccaA  5’-aTTattttTTTagTTaggagTaTaT  **Suppl. Table 1.** TXNIP primers used for bisulphite sequencing analysis on 5 CpG sequences on the TXNIP promoter region | 5 | 56 | chr1:145, 996, 678-145, 996, 891 |

*Generation of GFP and TXNIP-Transduced PB2B Cells*

This method has been previously described in Fredholm et al. [19]. Briefly, cDNA encoding TXNIP was synthesized and cloned into a 3rd-generation lentiviral vector pTRP-EGFP (provided by Dr. James L. Riley, University of Pennsylvania, Philadelphia, PA, USA) using 5’AvrII/3’SaII restriction sites (GeneArt/Thermo Fisher, Germany) generating the lentiviral vector pTRP-EGFP-TXNIP. This vector permits dual expression of EGFP and TXNIP from a single RNA transcript. The lentivirus was produced after transfection of HEK293T cells cultured in DMEM (BioWhittaker, Rockville MD, USA), 10% FBS, 100mg/ml P/S. Cells were seeded at 2.5 × cells/well in 6-well plates prior to transfection. For transfection, 1 µg of pTRP-EGFP-TXNIP and 0.5 µg of packaging and envelope plasmids (pTRP-RSV.Rev, pTRP-GAG-Pol and pTRP-VSVg) were used together with TurboFect Transfection Reagent (ThermoFisher Scientific). Cells were cultured for 48 h at 37ºC and 5% before harvesting the viral supernatant. For transduction of PB2B and MyLa2059, cells were incubated with lentiviral supernatant for 72 h before being sorted using a FACSAria cell sorter (BD Biosciences, San Jose, CA, USA). Both cell types were transduced with pTRP-EGFP accordingly.

*Flow Cytometry*

Bromodeoxyuridine (BrdU) incorporation assays were performed using the APC BrdU Flow Kit (BD Biosciences, San Diego, CA, USA) according to the supplier’s protocol. Briefly, transduced Myla2059 and PB2B cells were incubated in media containing RPMI-1640 supplemented with BrdU (17.7 μM) for 2 h. The cells were subsequently fixated and permeabilized, treated with DNase for 60 min at 37ºC and stained with APC anti-BrdU and 7-aminoactinomycin. The samples were finally analysed by flow cytometry on a FACS Calibur.