Cancer Letters 300 (2011) 20–29

Contents lists available at ScienceDirect

Cancer Letters

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

Transketolase-like protein 1confers resistance to serum withdrawal *in vitro*

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ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form 26 August 2010
Accepted 27 August 2010

Keywords:
TKTL1
Pentose phosphate pathway
Carcinoma

ABSTRACT

Transketolase-like protein 1 (TKTL1) is a member of the family of transketolase enzymes of which the founder member transketolase (TKT) is known to play a central role in the non-oxidative part of the pentose phosphate pathway. According to several publications TKTL1 is the only family member, whose expression is substantially de-regulated in a variety of solid tumours. Over-expression of TKTL1 correlates with poor prognosis of cancer patients and TKTL1 itself represents a potential therapeutic target owing to its possible involvement in the regulation of the proliferation and metabolism of cancer cells. We show that exogenously expressed TKTL1 provides HEK293 cells with moderate growth advantages under standard culture conditions, while protecting cells from growth factor withdrawal-induced apoptosis. Importantly, we identified TKTL1 with the JFC12T10 antibody as a 65 kDa protein, which was however absent in most tumour cell lines tested. Primary head and neck squamous cell carcinomas of various localisations were characterised by a focal pattern with single cells strongly expressing TKTL1, rather than by a homogeneous expression pattern within the tumour mass.

1. Introduction

Cancer cells are characterised by a high consumption of energy and heightened proliferation *in vitro* and *in vivo*. Consequently, cancer cells have an increased need for energy, for building blocks of RNA and DNA, *i.e.* pentoses, and for redox components to counteract the increased production of oxygen radicals. Especially pentoses are in high demand in order to fulfil the needs for DNA and RNA synthesis in rapidly dividing cells. Although less efficient, cancer cells commonly resort to glycolysis for the generation of ATP rather than to the mitochondrial oxidative phosphorylation. This enhanced glycolysis, which is characteristic of solid cancers and occurs even in the presence of sufficient oxygen concentrations, was termed the Warburg effect [1] or aerobic glycolysis. In parallel, the pentose phosphate pathway, as an alternative way to glycolysis for glucose degradation, additionally serves tumour cells to cover their requirements for pentoses. Accordingly, roughly 85% of pentoses incorporated in nucleic acids of tumour cells are products of the pentose phosphate pathway and, more precisely, of enzymatic reactions catalysed by transketolases [2,3].

Transketolases are vitamin B1-dependent enzymes and essential steps in the non-oxidative part of the pentose phosphate pathway are transketolase-dependent [4]. The transketolase family of enzymes is composed of the genuine transketolase (TKT) and two transketolase-like proteins (TKTL1 and 2). Functionally comparable, transketolases expedite the generation of (1) sedoheptulose-7-phosphate

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doi:10.1016/j.canlet.2010.08.017

and glyceraldehyde-3-phosphate out of ribose-5-phosphate and xylulose-5-phosphate, and of (2) fructose-6-phosphate and glyceraldehyde-3-phosphate out of xylulose-5-phosphate and erythrose-4-phosphate, and *vice versa* [5]. After its first description based on a robust homology to TKT [6], TKTL1 was demonstrated to be over-expressed in a variety of solid tumours such as head and neck [7], cervical [8], colon and urothelial cancers [9], breast cancer [10], and nephroblastomas [11]. Commonly, TKTL1 is a predictor of poor prognosis in these patients. Its potential role in the metabolism of rapidly proliferating cells and the over-expression in malignancies led to the notion that TKTL1 is a candidate proto-oncogene and potential therapeutic target [7,9,12–14].

Detection of TKTL1 was primarily achieved upon mRNA expression profiles [15], immunoblotting [7], or immunohistochemistry [11]. It is however noteworthy that the antibody in use has been shown to detect various forms of TKTL1, the molecular weight of which ranged from 92 kDa down to 25 kDa [4,13]. Those variants were assumed to represent full-length and C-terminal cleavage products of TKTL1. Using siRNA-mediated knock-down technologies and immunoblotting, we show that TKTL1 is a 65 kDa protein, which is actually absent or below the detection limit in most carcinoma cell lines tested, but strongly expressed in single cells within primary carcinomas. The function of TKTL1 as a moderate enhancer of proliferation under optimal culture conditions was confirmed. Importantly, exogenous expression of TKTL1 prevented induction of apoptosis following growth factor deprival. Hence, TKTL1 can in principle contribute to the malignant phenotype of tumour cells, however analyses of the expression patterns of TKTL1 might benefit from cautious revision.

2. Materials and methods

2.1. Cell lines, transfection, plasmid DNAs, and siRNA treatment

TKTL1 cDNA was cloned into the eukaryotic expression vector pCAG-141 to achieve a constitutive expression in HEK293 cells. For a control, pCAG was used without any cDNA insert. Stable cell clones were generated by transfection using the MATra reagent (IBA, Göttingen, Germany) and selection with 1 μg/ml puromycin (Sigma, Munich, Germany). HEK293 cell [16] transfectants were cultured in DMEM with 10% fetal calf serum and 1% antibiotics (penicillin/streptomycin). In order to inhibit TKTL1 expression the following siRNA oligonucleotides were used: 5′-GUCGUUUGUGGAUGUGGCA-3′ and 5′-UGCCACAUCCA CAAACGAC-3′ at a final concentration of 100 nM.

2.2. Flow cytometry and propidium iodide staining

Attached and floating cells were harvested, washed with PBS, and fixed in 70% ethanol. Subsequently, cells were incubated with RNase A (10U, Roche, Mannheim, Germany) and stained with propidium iodide (1 μg/ml) prior to analysis on a FACS flow cytometer (BD Clontech, Heidelberg, Germany). Apoptosis was determined by measuring the sub-genomic DNA content, which represented the DNA with a propidium iodide incorporation inferior to the G1-peak.

2.3. Immunohistochemistry and cytochemistry

THP-1, HeLa, PCI-1, PCI-13, 293pCAG, and 293pCAGTKTL1 cells were harvested, treated with paraformaldehyde and methanol, and incubated with the TKTL1 antibody JFC12T10 (R-Biopharm, Darmstadt, Germany). Afterwards cytospins were prepared on charged slides (Super Frost plus, Menzel, Braunschweig, Germany) for immunocytochemistry. After drying for 30 min at room temperature, slides were fixed in aceton for 10 min and incubated with a biotinylated secondary antibody followed by avidin–biotin-PO-complex in combination with the amino-ethylcarbazole substrate. Nuclei were counter-stained with hematoxylin. Specimens of different carcinomas and healthy tissue were shock frozen in liquid nitrogen and cut into 4 μm thick sections. Squamous cell carcinomas of larynx, hypopharynx (data not shown), and root of the tongue (data not shown) were additionally fixed in buffered formalin as well as in HOPE fixative [17] and thereafter embedded in paraffin. Immunohistochemistry was performed on formalin- or HOPE-fixed and paraffin-embedded and on cryo-preserved sections. For the case of formalin-fixed and paraffin-embedded tissue, sections were deparaffinised and antigen retrieval was carried out in 0.1 M citrate buffer (pH 6.0) in a 700-W microwave oven for up to 40 min. Alternatively, treatment with 0.1% pronase was conducted for 10 min at room temperature. HOPE-fixed tissue sections do not need any demasking treatment. The sections were incubated in 0.03% hydrogen peroxide (cryo-preserved sections) or 3% hydrogen peroxide (HOPE- and formalin-fixed sections) for 5 min to quench endogenous peroxidase. Tissue sections were then incubated with the monoclonal TKTL1 antibody JFC12T10 and the antigen detected with the avidin–biotin-PO-complex method. Immunohistochemical reactions were developed with amino-ethylcarbazole as the chromogenic substrate for peroxidase, and slides were counter-stained with hematoxylin. The 293pCAGTKTL1 cell line served as the positive control. Negative controls were performed for all cases and consisted of identically prepared slides that were treated with isotype mouse IgG2 instead of the primary TKTL1-specific antibody.

2.4. Cell numbers and doubling times

HEK293 transfectants were plated in 10 cm dishes at 5×10^5 cells/dish. Cell numbers were assessed at different time points with the trypan blue exclusion assay. Doubling times were calculated as described elsewhere [18].

2.5. RT-PCR and immunoblotting

Total RNA from cell lines (5×10^6 cells) was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was generated from 1 μg of total RNA using the reverse transcription system according to the manufacturer's instructions (Promega, Madison, US). Semi-quantitative