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Mini-review

# mRNA export protein THOC5 as a tool for identification of target genes for cancer therapy

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ABSTRACT

Recent evidence indicates that mRNA export is selective, giving priority to a subset of mRNAs that control diverse biological processes including cell proliferation, differentiation, stress response, and cell survival as well as tumor development. The depletion of a member of the mRNA export complex, the THO complex, impairs the expression of only a subset of genes, but causes dramatic changes in phenotype, such as cell cycle inhibition, abnormal differentiation, and importantly apoptosis of stem cells and cancer cells but not normal epithelial cells, hepatocytes, or fibroblasts.

Recent exosome sequence data revealed that over 100 driver gene mutations with a number of signaling pathways are involved in human cancer formation, indicating that multiple signaling pathways will need to be inhibited for cancer therapy. In this review we firstly describe a basic feature and function of the mRNA export complex, THO, secondly, the biological alteration upon depletion of a member of the THO complex in normal and cancer cells, and thirdly, identification of its target genes. Finally we describe our recent data on selection of targeting candidates from THOC5 dependent genes for application in cancer therapy.



## Introduction

All cancers are the result of somatic and/or germ line mutation. Recent data obtained from exosome sequencing has been used to analyze approximately 5 million mutations from over 7000 cancers [1]. A typical example is hepatocellular carcinoma (HCC), a frequent form of cancer with poor prognosis and with limited possibilities for medical intervention. It has been recently shown by exome sequencing of HCC that 161 putative driver genes are associated with 11 recurrently altered pathways in cancer development [2], suggesting that multiple pathways will need to be inhibited for any therapeutic method to be successful.

mRNA processing, including elongation, 3′ processing, splicing and mRNA export, is tightly regulated by a complex RNA-protein network that is essential for the maintenance of cellular and tissue homeostasis. Evidence has accumulated indicating that in cancer cells these proteins are dysregulated and pre-mRNAs are alternatively spliced and/or cleaved at the 3′-end. It has been clearly demonstrated that spliceosome-associated mutations have an effect on cancer development [3]. For example, it has been recently reported that splicing kinases are dysregulated and participate in tumorigenesis [4]. Furthermore, the driver mutation of genes that encode splicing factors [5], such as splicing factor 3B1 (SF3B1) or serine/arginine-rich splicing factor 2 (SRSF2) were found in several cancer cells [3]. Importantly, the mutations of RNA processing proteins, such as splicing factors or mRNA export proteins, influence the expression of a subset of genes. Thus, the depletion of RNA processing protein may be more effective than single molecule target agent. This review focuses on the potential of a member of an mRNA export complex as a tool for identifying target genes for cancer therapy.

### *Features and functions of an mRNA export complex, the THO complex*

The TREX (transcription/export) complex consists of a large number of proteins, such as members of the THO complex, ALY/REF export factor (ALYREF or THOC4), ATP-dependent RNA helicase UAP56, zinc finger CCCH-type containing 11A (ZC3H11A), polymerase (DNA-directed), delta interacting protein 3 (POLDIP3), chromatin target of PRMT1 (CHTOP), leucine zipper protein 4 (LUZP4), forty-two-three domain containing 1 (FYTTD or UIF) [6,7], and SAP domain containing ribonucleoprotein (SARNP or CIP29) [8]. TREX plays a key role in mRNA processing and export. The molecular function of each member of TREX has been intensively investigated (see Ref [9] for a comprehensive review of TREX). Notably, although ALYREF is also named THOC4, it has been shown

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**Table 1**
Overview of members of the human THO complex.

| Name | Amino acid number | Conserved domains [18] | Subcellular localization* | polyA accumulation in nuclei by depletion in HeLa cells [19] | Essential for optimal proliferation (p–value < 0.005) [20] |
|---|---|---|---|---|---|
| **THOC1** | 657 | Death–like domain | N | ++ | 2/4** |
| **THOC2** | 1593 | – | N | ++ | 2/4 |
| **THOC3** | 351 | WD40 | N | +/– | 0/4 |
| **THOC5** | 683 | PEST like domain [21] | N (C) [22] | +/– | 4/4 |
| **THOC6** | 341/296 | WD40 | N | +/– | 0/4 |
| **THOC7** | 204/152 | – | N/C [23] | ++ | 4/4 |

* N: Nucleus; C: cytoplasm.
** Essential for cell proliferation (number of positive cell lines out of 4 leukemic cell lines).

not to belong to the THO complex [10]. ALYREF and Luzp4 were recently shown to be expressed at high level in several human cancers [11], however it is not clear whether any other members of TREX beside THO complex participate in cancer development.

The THO complex, a subcomplex of TREX was originally identified in *Saccharomyces cerevisiae* as a five protein complex [12–14] that plays a role in transcriptional elongation, 3′processing, nuclear RNA export and genome stability. In higher eukaryotes such as *Drosophila melanogaster* [15] or humans [16], the THO complex is a six protein complex composed of THOC1, THOC2, THOC3, THOC5/Fms interacting protein (FMIP) [17], THOC6 and THOC7 (Table 1). Although the THO complex plays a role in mRNA processing, all members of this complex do not contain a feature that is typical of an RNA binding motif. In the yeast system, it has been shown that the C-terminal end of yeast THOC2 interacts with nucleic acids [24]. It is not clear, however, whether mammalian THOC2 directly interacts with RNA because the C-terminal domains of mammalian and yeast THOC2 are very different. THOC1 contains the death-like domain (Table 1). The function of this domain is still unclear. Other members of this complex, THOC3 and THOC6 contain exclusively the WD40 domain, and this 6- or 7-bladed beta propeller fold structure may play a role in the complex formation of THO. Mammalian THOC5 exhibits a PEST like domain that contains three ataxia-telangiectasia mutated (ATM) kinase specific phosphorylation sites [21] (Table 1). Although members of TREX, THOC1, THOC2, THOC3, THOC5, THOC6, THOC7, ALYREF and UAP56, were isolated from HeLa nuclei as one complex [16], it is not clear how and where this complex is formed. The subcellular distribution in MEF cells reveals that THOC7 is mainly in the cytoplasm, while THOC1 and THOC5 in the same cells are mainly in the nucleus (Table 1). The functional role of THOC7 in cytoplasm remains to be studied. Furthermore, depletion of each individual member of THO complex showed divergent results (Table 1) [19]. The depletion of THOC1, THOC2 and THOC7 in HeLa cells led to severe polyA+ RNA export inhibition, whereas THOC3, THOC5, and THOC6 depletion only had mild effects [19]. The authors of these studies suggest that one of these THO proteins might be the determinant factor for the mRNA export function of THO. Furthermore, recent large-scale genetic analysis [20] reveals that THOC5 and THOC7 are essential for cell proliferation in all four leukemic cell lines that were examined, while THOC3 and THOC6 are not (Table 1). Here, other members of TREX are not essential for leukemia proliferation except ALYREF (4 positive out of 4 leukemia cell lines) and UAP56 (1/4) [20].

### *Biological alteration upon depletion of a member of the THO complex in* in vivo *and* in vitro *systems*

#### In vivo *studies*

Among all members of the THO complex, only THOC1 and THOC5 knockout mice are presently available. The phenotype of both knockout mice was similar. Conventional knockout of both genes induces embryonic lethality and both THOC1 and THOC5 are required for the maintenance of hematopoietic and intestinal stem cells [25–28]. Interestingly, THOC5 is not required for the maintenance of mature organs, such as kidney or liver [25].

#### In vitro studies

Using an *in vitro* cell culture system it has been shown that THOC2 and THOC5 are required for self-renewal and somatic cell reprogramming of embryonic stem cells [14]. Furthermore, depletion of THOC5 in fibroblasts or macrophages suppressed cell proliferation. Importantly, certain cancer cells, such as HCC cells, HepG2 or Huh7, underwent apoptosis upon depletion of THOC5 [29]. In agreement with these data, depletion of THOC1 caused apoptosis in certain cancer cells, such as pancreas or prostate cancer, but not in normal fibroblasts [30,31]. These observations raised the question as to whether depletion of other members of the THO complex also induces apoptosis in cancer cells. Cells depleted of THOC1, THOC2, THOC5, THOC6 and THOC7, but not THOC3, underwent apoptosis (Fig. 1) [29], suggesting that these THOCs dependent genes may be useful candidates for targeting HCC. Of the various members of the THO complex, THOC5 has been most intensively studied in regard to identification of its target genes in several cell systems.

### *Knockdown of THOC5 influenced processing of a subset of mRNA species*

Global mRNA expression patterns after depletion of THOC5 have been examined in several cell systems (Table 2). In fibroblasts and bone marrow macrophages, processing of 143 and 99 mRNAs, respectively, were impaired upon depletion of THOC5 [32,34], (Table 2). Although depletion of THOC5 suppressed less than 0.5% of steady state mRNAs in fibroblasts, expression of 90% of serum induced immediate early genes were impaired in the absence of THOC5 [33]. Strikingly, depletion of THOC5 in embryonic stem cells downregulated 541 genes [14]. Furthermore, depletion of THOC5 in HCC cell lines Huh7 and HepG2 caused downregulation of 1248 and 836 genes [29], respectively. It should be noted that less than 5% of all genes were commonly downregulated in HCC cells and in ES cells, fibroblasts, or macrophages upon depletion of THOC5. In human cervical cancer, HeLa cells, however, 20% of downregulated genes were common in HCC.

Importantly, THOC5 expression was enhanced in 78% of cytological differentiation grading G2 and G3 primary HCC compared to normal hepatocytes [29] (Fig. 2A). These data imply that suppression of multiple THOC5 target genes may represent a novel strategy for HCC therapy.

### *THOC5 dependent genes: potential target genes for cancer therapy*

Since enhanced expression of THOC5 was observed in primary HCC [29], the HCC system was chosen as a model system for the identification of target genes for cancer therapy.