ISSN 0025-7680

International Symposium
NEW DIRECTIONS IN CANCER MANAGEMENT
Academia Nacional de Medicina
Buenos Aires, 6-8 June 2007

MEDICINA (Buenos Aires) 2007; 67 (Supl. II): 13-19

Thoc1 AS A POTENTIAL MOLECULAR TARGET FOR CANCER THERAPY

**YANPING LI, ATHENA W. LIN, XIAOJING ZHANG, YANQING WANG,
XIAOLING WANG, DAVID W. GOODRICH**

Roswell Park Cancer Institute, Buffalo NY, USA

Abstract The evolutionarily conserved TREX complex physically couples transcription, mRNP biogenesis, RNA processing, and RNA export for a subset of genes. *HPR1* encodes an essential component of the *S. cerevisiae* TREX complex. *HPR1* loss compromises transcriptional elongation, nuclear RNA export, and genome stability. Yet, *HPR1* is not required for yeast viability. Human *Thoc1* protein (pThoc1), originally discovered by its ability to bind an amino terminal domain of the *Rb1* tumor suppressor protein, is a functional orthologue of yeast *HPR1*. We will present evidence that suggests that pThoc1 may be a good molecular target for cancer therapy. *Thoc1* protein levels increase upon functional inactivation of *Rb1*. Consistent with this observation, pThoc1 is also expressed at higher levels in cancer cells than in normal cells. *Thoc1* expression levels tend to correlate with poorer clinical outcome. Depletion of *Thoc1* protein (pThoc1) compromises the viability of cancer cells by induction of apoptotic cell death. Induction of apoptosis is coincident with increased DNA damage as indicated by the appearance of phosphorylated histone H2AX. In contrast, the viability of normal cells is largely unaffected by pThoc1 loss. Further, normal cells lacking *Thoc1* cannot be transformed by forced expression of E1A and Ha-*ras,* suggesting that *Thoc1* may be a requirement for neoplastic transformation. In sum, our data are consistent with the hypothesis that cancer cells require higher levels of pThoc1 for survival than normal cells. If true, pThoc1 may provide a novel molecular target for cancer therapy. Possible advantages of utilizing pThoc1 as a molecular target for cancer therapy are discussed.

Key words: TREX complex, Thoc1, cancer therapy

Resumen ***Thoc1 como potencial blanco molecular en la terapia contra el cáncer.*** El complejo evolutivamente conservado TREX acopla físicamente la transcripción, la biogénesis mRNP, el procesamiento del RNA, y la exportación de RNA para un grupo de genes. HPR1 codifica para un componente esencial del complejo TREX de *S. cerevisiae*. La pérdida de HPR1 compromete la elongación transcripcional, la exportación nuclear del RNA y la estabilidad genómica. Sin embargo, HPR1 no es necesaria para la viabilidad de levaduras. La proteína humana *Thoc1* (pThoc1), originalmente descubierta por su habilidad de unir el dominio amino terminal del gen supresor de tumor *Rb1*, es un ortólogo funcional de HPR1 de levaduras. Presentamos evidencias que sugieren que pThoc1 podría ser un buen blanco molecular para el tratamiento del cáncer. Los niveles de la proteína *Thoc1* se incrementan con la inactivación funcional de *Rb1.* Consistente con esta observación, pThoc1 se expresa en mayores niveles en células tumorales que en normales. La expresión de *Thoc1* correlaciona con un peor pronóstico clínico. La depleción de pThoc1 compromete la viabilidad de células tumorales por inducción de muerte celular por apoptosis. La inducción de apoptosis coincide con un incremento del daño al DNA como lo indica la aparición de la histona H2AX fosforilada. Por el contrario, la viabilidad de células normales prácticamente no está afectada por la pérdida de pThoc1. Más aún, células normales que carecen de *Thoc1* no pueden ser transformadas por la expresión forzada de E1A y Ha-*ras*, sugiriendo que *Thoc1* podría ser requerida para la transformación neoplásica. En resumen, nuestros datos son consistentes con la hipótesis de que células tumorales requieren de niveles más elevados de pThoc1 para sobrevivir que células normales. Si esto resulta cierto, pThoc1 podría ser un nuevo blanco para la terapia del cáncer. Las posibles ventajas de utilizar a *Thoc1* como blanco molecular son discutidas en este trabajo.

Palabras clave: complejo TREX, Thoc1, terapia del cáncer

The accumulation of genetic and epigenetic alterations in cancer cells endows them with unwanted proliferative and metastatic potential. However these alterations also handicap cancer cells with unique vulnerabilities such that it is possible to identify genes whose function is more critical for the viability of cancer cells than for normal cells[1]. Mutations in such genes are formally synthetic lethal with the genetic and epigenetic alterations present in cancer cells. Proteins encoded by such synthetic lethal genes identify molecular targets for therapy since antagonizing their function will be more toxic to cancer cells than to normal cells, thus yielding superior therapeutic index. The inability to perform synthetic lethal screens in cultured human cells has limited the number of genes identified whose inactivation is uniquely toxic to human cancer cells.

- - - -
e-mail: david.goodrich@roswellpark.org

The human *Thoc1* gene, also known as hHpr1 or p84, encodes a protein that was originally identified as a nuclear matrix (NM) component that binds the retinoblastoma tumor suppressor protein[2]. Alterations in the NM and resulting changes in nuclear structure have long been recognized to correlate with tumor progression, prompting their use as biomarkers for the diagnosis of cancer[3]. Indeed, overexpression of *Thoc1* has recently been documented in human breast cancer with pThoc1 levels correlating with tumor size and metastases[4]. We and others have recently identified pThoc1 as a functional orthologue of the *S. cerevisiae HPR1* gene[4-6]. The *HPR1*-encoded protein (Hpr1p) is a component of the TREX (Transcription/Export) complex that physically couples the elongating RNA polymerase II with factors important for mRNP formation, RNA processing, and nuclear RNA export[7-9]. Yeast TREX is composed of the salt resistant THO subcomplex containing the four proteins Hpr1p, Tho2p, Mft1p, and Thp2p, all of which are essential for efficient transcriptional elongation of a subset of yeast genes[10]. THO associates with two proteins involved in nuclear RNA export, Sub2p and Yra1p, to form the larger TREX complex[9]. Sub2p is the yeast orthologue of human UAP56 that has been implicated in RNA splicing[11]. Hpr1p genetically and physically interacts with RNA PolII[12, 13] and is essential for recruitment of Sub2p to genes regulated by TREX[14]. Loss of Hpr1p impairs both transcriptional elongation and nuclear RNA export[4, 8, 9, 15-18]. While Hpr1p is an essential component of the TREX complex, it is not essential for yeast viability[19].

The human TREX complex is composed of proteins encoded by *Thoc1* (yeast *HPR1*), *Thoc2* (yeast *THO2*)[20], *hTex1* (yeast *TEX1*), *UAP56* (yeast *SUB2*)[21], *Aly* (yeast *YRA1*)[22], and other gene products that are not evolutionarily conserved with yeast[4, 6]. Depletion of pThoc1 compromises both transcriptional elongation and cell proliferation in the human cancer cell lines that have been tested[4, 5]. However the effects of pThoc1 depletion on normal mammalian cells, both in vitro and in vivo, have not been tested. Thus it is possible that, in contrast to yeast, *Thoc1* is essential for mammalian cell viability. Alternatively, cancer cells may be uniquely dependent on Thoc1 such that it is not required for normal cell viability. We present results from experiments designed to distinguish between these possibilities using both *in vitro* and *in vivo* model systems.

Results

Loss of Thoc1 compromises the viability of oncogene-transformed fibroblasts, but not normal fibroblasts, cultured in vitro. We have previously generated oncogene transformed derivatives of normal, diploid IMR90 human fibroblasts[23]. An *E1A/Ha-ras* transduced IMR90 derived line is immortal, but not tumorigenic. An *E1A/Ha-ras/c-myc* transduced IMR90 line is immortalized and tumorigenic *in vivo*. We have used this panel of human fibroblast cells to compare the effects of pThoc1 depletion on normal and oncogene transformed human cells. Examination of pThoc1 levels in the cell panel indicates that the oncogene transformed lines express significantly higher levels of pThoc1 than the normal, parental cells.

We transfected siRNA directed against *Thoc1* into each cell line to deplete pThoc1 and assessed the effects on accumulation of viable cells over time. *Thoc1* siRNA, but not a mismatch control siRNA, was able to deplete pThoc1 from each of the cell lines tested. To verify that siRNA mediated pThoc1 depletion inhibited TREX activity, we assayed the expression of a bacterial β galactosidase reporter gene. *Thoc1* was previously noted to be required for efficient transcriptional elongation of the G/C rich bacterial β-galactosidase gene in both yeast and mammalian cells[5, 16]. Cells transfected with *Thoc1* or control siRNA were infected with a recombinant, β-galactosidase expressing adenovirus and then assayed for β galactosidase activity. Consistent with western blot analysis, *Thoc1* siRNA treatment reduced β-galactosidase activity in all tested cell lines relative to cells transfected with control siRNA. Loss of pThoc1 in parental IMR90 cells had no detectable effect on cell accumulation compared to control siRNA treated cells. In contrast, *Thoc1* siRNA caused a significant decrease in the accumulation of *E1A/Ha-ras or EIA/Ha-ras/c-myc* transformed IMR90 cells.

We investigated whether the decrease in the accumulation of transformed cells might be due to induction of apoptotic cell death since a significant number of pThoc1 depleted cells stained positively for trypan blue. *Thoc1* siRNA treatment of *EIA/Ha-ras/c-myc* transformed cells caused an approximately two-fold increase in the fraction of cells staining positively for the apoptosis marker annexin V, relative to control siRNA treated cells. Thoc1 siRNA treatment had no detectable effect on apoptosis in IMR90 cells. Similarly, *Thoc1* siRNA caused a significant increase in the fraction of *EIA/Ha-ras/c-myc* transformed cells containing fragmented DNA, as assessed by TUNEL assay, relative to control siRNA treated cells. We also examined whether there were changes in cell cycle distribution upon pThoc1 loss. No significant difference in cell cycle distribution was observed in either IMR90 or *EIA/Ha-ras/c-myc* transformed cells upon pThoc1 depletion. These data suggested that loss of cell accumulation in transformed cells was due to apoptotic cell death rather than changes in the cell cycle, and that oncogene transformed human fibroblasts were significantly more sensitive to pThoc1 depletion than normal human fibroblasts.

We have investigated two additional normal, diploid human fibroblast cell lines to ensure that insensitivity to pThoc1 depletion was not unique to IMR90 cells. *Thoc1*

siRNA efficiently depleted pThoc1 in both WI38 and IMR5C normal diploid human fibroblasts, yet loss of pThoc1 had no detectable effect on the growth rate of these cells in culture. Coincident with the decrease in *Thoc1* protein levels, *Thoc1* siRNA treatment also decreased the level of β-galactosidase activity by more than two-fold compared to control siRNA transfected cells. Thus efficient depletion of pThoc1 protein and TREX activity had no detectable effect on the growth of normal human fibroblasts *in vitro*.

We have recently generated a conditionally excisable allele of the murine *Thoc1* gene that facilitates stable depletion of pThoc1[24]. To test the effects of complete genetic loss of Thoc1, relative to intermediate levels of depletion achievable by siRNA, and to ensure that the results described above were not caused by off-target effects of siRNA mediated gene silencing, we have used murine embryonic fibroblasts (MEF) containing the conditional *Thoc1* allele to compare the effects of *Thoc1* loss on the viability of normal and transformed cells.

Early passage (<5) MEFs from littermate embryos heterozygous (*Thoc1*$^{F/+}$) or hemizygous (*Thoc1*$^{F/-}$) for the conditional allele were transformed into neoplastic cells by retroviral mediated transfer of the *E1A* and *Ha-ras* oncogenes[25]. Both *Thoc1*$^{F/+}$ and *Thoc1*$^{F/-}$ MEFs were efficiently transformed by *E1A/Ha-ras* as indicated by the large number of drug selection resistant cell colonies that exhibited classic transformed morphology. To test whether *Thoc1* is required for the viability of these oncogene transformed cells, we infected them with a Cre expressing recombinant adenovirus (AdCre) to convert the *Thoc1*^{F} allele to a null allele. AdCre caused a significant reduction in the accumulation of *Thoc1*$^{F/-}$ transformed cells, but not of *Thoc1*$^{F/+}$ transformed cells. We have so far failed to recover viable, *E1A/Ha-ras* transformed cell clones that lack pThoc1. We also investigated whether normal, primary MEFs required *Thoc1* for viability. Early passage MEFs isolated from *Thoc1*$^{F/-}$ and *Thoc1*$^{F/+}$ mice were treated with AdCre and monitored for accumulation of viable cells. In contrast to *E1A/Ha-ras* transformed cells, there was no statistically significant difference in the relative accumulation of *Thoc1*$^{F/-}$ or *Thoc1*$^{F/+}$ cells subsequent to AdCre infection. As above, to test whether oncogene transformed MEFs underwent apoptosis upon *Thoc1* loss, we treated cells with AdCre and assayed for induction of apoptosis by annexin V staining. AdCre caused a significant increase in the percentage of apoptotic cells in transformed *Thoc1*$^{F/-}$ cell cultures, but not in transformed *Thoc1*$^{F/+}$ cell cultures that retain a wild type *Thoc1* allele. We did not observe significant apoptosis in primary MEFs of either genotype subsequent to AdCre treatment.

AdCre infection efficiently depleted pThoc1 from both primary and *E1A/Ha-ras* transformed *Thoc1*$^{F/-}$ cells analyzed above. As expected, AdCre treatment had no detectable effect on pThoc1 levels in either primary or *E1A/Ha-ras* transformed *Thoc1*$^{F/+}$ cells since they retain a wild type *Thoc1* allele. Thus despite equally efficient pThoc1 depletion, induction of apoptosis and loss of viability only occurred at detectable levels in oncogene transformed fibroblasts, not normal MEFs. We also noted that *E1A/Ha-ras* transformed MEFs expressed significantly higher levels of pThoc1 than primary MEFs of the same genotype. Consistent with the results from the human fibroblats, this data suggested that cancer cells may be dependent on higher levels of pThoc1 for survival than normal cells.

If true, *Thoc1* may be required for neoplastic transformation. To test this possibility, we have attempted to transform early passage primary *Thoc1*$^{F/-}$ and *Thoc1*$^{F/+}$ MEFs with *E1A/Ha-ras* subsequent to AdCre mediated excision of the *Thoc1* floxed allele. We have verified excision of the floxed allele by western blot analysis of MEF protein extracts prior to *E1A/Ha-ras* transduction. As expected, *Thoc1*$^{F/-}$ MEFs treated with AdCre exhibit significant depletion of pThoc1 relative to mock treated controls. *Thoc1*$^{F/+}$ MEFs, both Cre or mock pretreated, generate numerous colonies of morphologically transformed cells upon retroviral mediated *E1A/Ha-ras* gene transfer and drug selection. Mock treated *Thoc1*$^{F/-}$ MEFs from two independent MEF isolates also generate abundant transformed colonies upon transduction of *E1A/Ha-ras.* However, very few transformed cell colonies are recovered from *Thoc1*$^{F/-}$ primary MEFs lacking pThoc1. The few transformed colonies that are recovered have escaped AdCre mediated excision of *Thoc1* since all of those cell clones tested still express pThoc1. Similarly, *Thoc1*$^{F/-}$ primary MEFs treated with Cre and spontaneously immortalized by continuous *in vitro* culture under the 3T3 protocol also retain pThoc1. We have been unable to isolate any *E1A/Ha-ras* transformed or spontaneously immortalized cell clones that lack pThoc1. These observations suggest that *Thoc1* is required for spontaneous immortalization and *E1A/Ha-ras* mediated transformation of primary MEFs.

The data presented above demonstrated that neoplastic fibroblasts are more dependent on pThoc1 for survival than normal fibroblasts, and that neoplastic fibroblasts deprived of pThoc1 die via apoptotic cell death. We wished to determine whether human epithelial cancer cell lines also undergo apoptotic cell death upon pThoc1 loss, and to examine possible mechanisms that might trigger this apoptotic response. Thoc1 siRNA efficiently mediated depletion of pThoc1 in a number of different human cancer cell lines including HeLa, 293, HCT116, a p53 null derivative of HCT116, and MCF-7 cells. Depletion of pThoc1 reduced the accumulation of cells in each of these lines. To ascertain if pThoc1 loss also induced apoptotic cell death in human epithelial cancer cells, we assayed pThoc1 depleted HeLa cells for apoptosis. HeLa cells depleted of pThoc1 and exhibiting the typical two-fold reduction in cell accumulation showed

a greater than two-fold increase in the percentage of cells stained for the apoptosis marker annexin V, relative to control siRNA transfected cells. Increased apoptosis was verified by assaying cleavage of PARP, a known substrate of caspase proteases, in siRNA transfected HeLa cells. Specific depletion of pThoc1 caused an increase in PARP cleavage. Thoc1 siRNA treated HeLa, MCF-7, and HCT116 cells also showed an increase in the fraction of cells positive in the TUNEL assay relative to control siRNA treated cells. Finally, treatment of pThoc1 depleted HeLa cells with the pan-caspase inhibitor ZVAD diminished the fraction of annexin V positive cells. In sum, these data indicated that depletion of pThoc1 in human epithelial cancer cell lines inhibited proliferation by induction of apoptosis and subsequent loss of cell viability.

It should be noted that sensitivity to loss of pThoc1 was independent of p53 and caspase-3 status. HCT116 cells that retain wild type p53 and a p53 null HCT116 derivative cell line were both sensitive to Thoc1 siRNA. We also failed to observe consistent increases in p53 and p21 levels in pThoc1 depleted HeLa cells, suggesting that the p53 pathway was not activated. MCF-7 cells that lack caspase-3 were also sensitive to Thoc1 siRNA, and we did not detect caspase-3 activation upon pThoc1 depletion.

How pThoc1 deprivation triggers apoptotic cell death in cancer cells is currently unknown, but a number of potential mechanisms are conceivable. Loss of pThoc1 compromises transcriptional elongation due to improper incorporation of nascent RNA into mRNPs. Nascent RNA can then hybridize to the single stranded DNA template emerging from the RNA polymerase complex thus generating stable RNA:DNA R-loop structures that are known to be recombinagenic and may compromise DNA integrity[17]. *Thoc1 l*oss, therefore, may directly lead to DNA damage. To test this possibility, we have examined the accumulation of phosphorylated histone H2AX, a known marker for DNA damage, in pThoc1 depleted normal and cancer cells. Phosphorylated histone H2AX is undetectable in *Thoc1* or control siRNA treated normal IMR90 cells. In contrast, *Thoc1* siRNA treatment increases the levels of phosphorylated histone H2AX in *EIA/Ha ras/c* myc transformed IMR90 cells as well as in HeLa cells, compared to control siRNA treated cells. These results are consistent with the possibility that Thoc1 loss may lead to accumulation of DNA damage.

Effects of Thoc1 loss in vivo. The effects of Thoc1 loss on the physiology of metazoan species are largely undefined. To address this gap, we have created a series of mutant Thoc1 alleles in the mouse[24, 26]. The constitutive null allele contains a deletion of Thoc1 that removes evolutionarily conserved coding sequence and creates a reading frame shift such that no pThoc1 can be detected by western blot analysis. Heterozygous null Thoc1 mice are born at the expected Mendelian frequency with no phenotype distinguishable from wild type. In contrast, homozygous null mice are not recovered, indicating Thoc1 is required for embryonic development. Embryonic development is arrested around the time of implantation as blastocysts exhibit hatching and blastocyst outgrowth defects upon *in vitro* culture. Cells of the inner cell mass are particularly dependent on Thoc1 as these cells rapidly lose viability coincident with Thoc1 protein loss.

The early embryonic lethality of Thoc1 null mice precluded analysis of the effects of Thoc1 deficiency on tissues of the adult mouse. To circumvent this limitation, we created both hypomorphic and conditionally null alleles of Thoc1. The mutant *Thoc1* alleles were engineered by targeted homologous recombination in mouse ES cells such that a PGK-neo selection cassette flanked by frt sites was inserted within intron 5 in reverse orientation relative to *Thoc1*. LoxP sites flanked exons six and seven. The hypomorphic allele retained the PGK-neo selection cassette while the conditional allele had the cassette removed by Flp mediated recombination, leaving only the loxP sites. These loxP sites could be used to create a null allele by Cre mediated deletion of exons 6 and 7.

Mice heterozygous or homozygous for the hypomorphic allele (H allele) were born at a frequency not significantly different from the expected Mendelian ratio ($p>0.05$ by Chi squared test). *Thoc1* H/+ mice were indistinguishable from wild type, but *Thoc1* H/H mice exhibited a dwarf phenotype with 100% penetrance. The weight of newborn *Thoc1* H/H mice was less than mice containing at least one wild type allele (1.1g±0.1 *versus* 1.6±0.1, $P<0.01$ by Student's T test). Reduced size persisted throughout adulthood. By four months of age, total body weight of H/H mice was approximately 80% of wild type and most individual organ weights (thymus, heart, lung, liver, kidney, spleen, and urogenital tract) were also proportionally less. The relatively small size of H/H mice was detectable as early as embryonic day 12.5 (E12.5), suggesting the dwarf phenotype was not solely due to changes in the activity of growth factors or hormones that function during postnatal development. Viable neonates hemizygous for the *Thoc1* H allele (H/-) were not recovered. However, hemizygous embryos were detected at E13.5, thus reaching a stage of embryonic development significantly more advanced than *Thoc1* null embryos[26]. As with H/H embryos, H/- embryos were considerably smaller than their wild type littermates.

In addition to the dwarf phenotype, H/H male and femalie mice are both sterile. Sterility is due to defects in gametogenesis. Both spermatogenesis and oogenesis is interrupted during meiosis I with subsequent loss in the viability of affected germ cells. Thus males show a severe reduction in the number of morphologically mature sperm while females show a reduction in mature oocytes. To ascertain whether the phenotypes observed are due to reduced pThoc1 expression, we have isolated tissue or embryonic fibroblasts (MEF) from mice of informative

genotypes and assessed pThoc1 levels by western blotting. In multiple tissues pThoc1 levels are significantly lower in *Thoc1* H/H mice than in wild type or H/+ mice. The relative decrease in pThoc1 levels ranges from more than 30-fold in brain tissue, to approximately 30% in lung tissue. Most H/H tissues and MEFs show a 2-4 fold decrease in pThoc1 levels relative to samples from wild type or heterozygous mice. Other than the phenotypes noted, H/H male and female mice appear normal.

The phenotype of the hypomorphic mice suggest the hypothesis that normal levels of pThoc1 are not required for the proper differentiation and function of most tissues. However, variable levels of reduction in pThoc1 levels in the various tissues of H/H mice complicate interpretation of the data. To more stringently test the requirement for *Thoc1* in the development and function of a single tissue, we have bred mice homozygous for the conditional floxed allele and containing either the MMTV-Cre or WAP-Cre transgenes. Both MMTV-Cre and WAP-Cre transgenic mice express Cre primarily in the mammary epithelia. Interestingly, conditional ablation of *Thoc1* in adult mammary epithelia in these mice has no detectable effect on gland function. Homozygous floxed *Thoc1* female mice containing either the MMTV-Cre or WAP-Cre transgenes nurse their offspring normally. The morphology and differentiation of the glands appears normal by histological and immunohistochemical analysis. In sum, the phenotypes of the hypomorphic and conditional null *Thoc1* mice indicate that most mouse tissues can tolerate depletion of pThoc1 *in vivo*. Current ongoing studies are designed to test whether pThoc1 depletion affects tumorigenesis in vivo.

Discussion

We describe a number of observations that suggest that cancer cells have a unique requirement for *Thoc1*. *Thoc1* expression is higher in oncogene transformed human and mouse fibroblasts compared to normal fibroblasts. Thoc1 expression is also higher in mouse and human prostate cancer specimens compared to normal prostate epithelium. This mimics the increased *Thoc1* expression previously observed in primary human breast cancer compared to normal mammary epithelium[4], and the abundant levels of pThoc1 typically observed in human cancer cell lines that have been tested[5]. However, higher pThoc1 levels are not merely a reflection of the faster proliferation rate typical of cancer cells. Loss of pThoc1 in cancer cell lines and oncogene transformed human or mouse fibroblasts inhibit cell accumulation through induction of apoptosis and subsequent loss in cell viability. In contrast, loss of pThoc1 in normal human or mouse fibroblasts has no detectable effect on viability. Depletion or loss of *Thoc1* in vivo has little effect on the development and function of most tissues. Thus the requirements for *Thoc1* to support viability are different in normal and cancer cells. We have been unable to recover viable, *E1A/Ha-ras* transformed or spontaneously immortalized MEFs that lack pThoc1. Thus cells undergoing neoplastic transformation or immortalization are unable to adapt to the absence of *Thoc1*, suggesting *Thoc1* may be required.

We have observed that *Thoc1* is required for the viability of blastocyst stage mouse embryos[26]. Normal levels of pThoc1 are also required for gametogenesis in both male and female mice homozygous for the hypomorphic *Thoc1* allele. Blastocyst stage embryos are comprised largely of stem or progenitor cells that, like cancer cells, have an extended potential for replication and self-renewal. Similarly, germ cells must maintain indefinite replicative potential, particularly in males, in order to supply a continuous supply of gametes. Thus, *Thoc1* may be required more generally for the maintenance of extended replicative potential. Consistent with this hypothesis, we have observed that primary MEFs genetically ablated for *Thoc1*, while viable and able to proliferate in culture, undergo premature conversion to a senescence-like state. Similarly, loss of the yeast orthologue *HPR1* is not lethal, but compromises lifespan[27]. It is conceivable that lack of sufficient pThoc1 limits replicative potential in normal cells by induction of a cellular senescence program, thereby inhibiting immortalization and neoplastic transformation. Upregulation of *Thoc1* may, therefore, be required to facilitate immortalization and neoplastic transformation. Since cancer cells are unable to sustain cell viability upon acute loss of pThoc1 activity, the effects of pThoc1 loss must be dominant to the effects of deregulated *E1A/Ha-ras* expression studied here, as well as to other alterations that facilitate neoplastic transformation in a variety of different human cancer cell lines.

How *Thoc1* loss triggers apoptosis in cancer cells is unknown. Since all detectable pThoc1 is apparently within the TREX complex[6], the simplest explanation is that loss of pThoc1 compromises TREX function. Loss of TREX function could have several conceivable effects on cells. Loss of TREX activity may adversely affect the generation of translatable mRNA from a subset of genes required to maintain viability. Loss of TREX activity may also compromise normal telomere maintenance. In yeast, loss of the *Thoc1* orthologue *HPR1* is associated with defects in telomere maintenance[28]. Defects in telomere maintenance would be expected to influence replicative potential and possibly viability. Alternatively, deficient mRNP biogenesis in the absence of *Thoc1* may trigger R loop formation and DNA strand breaks[29]. Such DNA lesions could trigger apoptotic cell death in cancer cells if they are unable to efficiently repair them. The accumulation of phosphorylated histone H2AX upon pThoc1 depletion observed here is consistent with this possibility.

Irrespective of the precise mechanism, mutational inactivation of *Thoc1* is synthetic lethal with the genetic and

epigenetic alterations associated with a number of cancer cell lines of different type and origin. Thus *Thoc1* may represent a novel molecular target for cancer therapy. Therapy that blocks pThoc1 activity is expected to preferentially compromise the viability of cancer cells, potentially yielding superior therapeutic index. Since the mechanism of pThoc1 action is novel, utilization of pThoc1 as a therapeutic target may yield unique clinical responses and opportunities for novel combination therapy. For example, yeast deficient in the *Thoc1* orthologue *HPR1* are synthetic lethal with topoisomerase mutations[30] and are more sensitive to DNA damage[31]. Depletion of pThoc1 in human cancer cell lines renders them more sensitive to camptothecin and cisplatin[5]. These observations suggest that therapeutic inhibition of pThoc1 in human cancer will increase sensitivity to topoisomerase poisons and possibly other forms of genotoxic therapy.

Thoc1 protein functions in the newly discovered TREX complex, a representative of a class of complexes that regulate gene expression subsequent to transcriptional initiation. This class of protein complexes may specify post transcriptional "operons" that facilitate protein expression from coordinately regulated genes of diverse size and structure[32]. Although there is increasing appreciation for the importance of such complexes, they are understudied relative to the transcription factors that govern the initiation of transcription. As such, their relevance to carcinogenesis is largely undocumented. However, the von Hippel-Lindau tumor suppressor protein is a known inhibitor of the elongin transcription elongation factor[33]. The interaction of the retinoblastoma tumor suppressor protein and pThoc1 may reflect an analogous interaction between a tumor suppressor gene product and a transcription elongation/RNA processing factor. Such interactions suggest that complexes that regulate gene expression at the level of mRNP formation and RNA processing may provide a largely untapped source of novel molecular targets for cancer therapy.

References

1. Kaelin WG, Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 2005; 5: 689-98.
2. Durfee T, Mancini MA, Jones D, Elledge SJ, Lee WH. The amino-terminal region of the retinoblastoma gene product binds a novel nuclear matrix protein that co-localizes to centers for RNA processing. *J Cell Biol* 1994; 127: 609-22.
3. Zink D, Fischer AH, Nickerson JA. Nuclear structure in cancer cells. *Nat Rev Cancer* 2004; 4: 677-87.
4. Guo S, Hakimi MA, Baillat D, et al. Linking Transcriptional Elongation and Messenger RNA Export to Metastatic Breast Cancers. *Cancer Res* 2005; 65: 3011-6.
5. Li Y, Wang X, Zhang X, Goodrich DW. Human hHpr1/ p84/Thoc1 Regulates Transcriptional Elongation and Physically Links RNA Polymerase II and RNA Processing Factors. *Mol Cell Biol* 2005; 25: 4023-33.
6. Masuda S, Das R, Cheng H, Hurt E, Dorman N, Reed R. Recruitment of the human TREX complex to mRNA during splicing. *Genes Dev* 2005; 19: 1512-7.
7. Abruzzi KC, Lacadie S, Rosbash M. Biochemical analysis of TREX complex recruitment to intronless and intron-containing yeast genes. *EMBO J* 2004; 23: 2620-31.
8. Rondon AG, Jimeno S, Garcia-Rubio M, Aguilera A. Molecular evidence that the eukaryotic THO/TREX complex is required for efficient transcription elongation. *J Biol Chem* 2003; 278: 39037-43.
9. Strasser K, Masuda S, Mason P, et al. TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* 2002; 417: 304-8.
10. Chavez S, Beilharz T, Rondon AG, et al. A protein complex containing Tho2, Hpr1, Mft1 and a novel protein, Thp2, connects transcription elongation with mitotic recombination in Saccharomyces cerevisiae. *EMBO J* 2000; 19: 5824-34.
11. Luo ML, Zhou Z, Magni K, et al. Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and Aly. *Nature* 2001; 413: 644-7.
12. Chang M, French-Cornay D, Fan HY, Klein H, Denis CL, Jaehning JA. A complex containing RNA polymerase II, Paf1p, Cdc73p, Hpr1p, and Ccr4p plays a role in protein kinase C signaling. *Mol Cell Biol* 1999;19:1056-67.
13. Fan HY, Cheng KK, Klein HL. Mutations in the RNA polymerase II transcription machinery suppress the hyperrecombination mutant hpr1 delta of Saccharomyces cerevisiae. *Genetics* 1996; 142: 749-59.
14. Zenklusen D, Vinciguerra P, Wyss JC, Stutz F. Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p. *Mol Cell Biol* 2002; 22: 8241-53.
15. Chavez S, Aguilera A. The yeast HPR1 gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. *Genes Dev* 1997; 11: 3459-70.
16. Chavez S, Garcia-Rubio M, Prado F, Aguilera A. Hpr1 is preferentially required for transcription of either long or G+C-rich DNA sequences in Saccharomyces cerevisiae. *Mol Cell Biol* 2001; 21: 7054-64.
17. Huertas P, Aguilera A. Cotranscriptionally formed DNA: RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. *Mol Cell* 2003; 12: 711-21.
18. Rehwinkel J, Herold A, Gari K, et al. Genome-wide analysis of mRNAs regulated by the THO complex in Drosophila melanogaster. *Nat Struct Mol Biol* 2004; 11: 558-66.
19. Aguilera A, Klein HL. HPR1, a novel yeast gene that prevents intrachromosomal excision recombination, shows carboxy-terminal homology to the Saccharomyces cerevisiae TOP1 gene. *Mol Cell Biol* 1990; 10: 1439-51.
20. West RW, Jr., Kruger B, Thomas S, Ma J, Milgrom E. RLR1 (THO2), required for expressing lacZ fusions in yeast, is conserved from yeast to humans and is a suppressor of SIN4. *Gene* 2000; 243: 195-205.
21. Stutz F, Bachi A, Doerks T, et al. REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. *RNA* 2000; 6: 638-50.
22. Strasser K, Hurt E. Yra1p, a conserved nuclear RNA-binding protein, interacts directly with Mex67p and is required for mRNA export. *EMBO J* 2000; 19: 410-20.
23. Mason DX, Keppler D, Zhang J, et al. Defined genetic events associated with the spontaneous in vitro transformation of EIA/Ras-expressing human IMR90 fibroblasts. *Carcinogenesis* 2006; 27: 350-9.
24. Wang X, Li Y, Zhang X, Goodrich DW. An allelic series

for studying the mouse Thoc1 gene. *Genesis* 2007; 45: 32-7.
25. Hunter T. Cooperation between oncogenes. *Cell* 1991; 64: 249-70.
26. Wang X, Chang Y, Li Y, Zhang X, Goodrich DW. Thoc1/Hpr1/p84 is essential for early embryonic development in the mouse. *Mol Cell Biol* 2006; 26: 4362-7.
27. Merker RJ, Klein HL. hpr1Delta affects ribosomal DNA recombination and cell life span in Saccharomyces cerevisiae. *Mol Cell Biol* 2002; 22: 421-9.
28. Askree SH, Yehuda T, Smolikov S*, et al.* A genome-wide screen for Saccharomyces cerevisiae deletion mutants that affect telomere length. *Proc Natl Acad Sci USA* 2004; 101: 8658-63.
29. Aguilera A. mRNA processing and genomic instability. *Nat Struct Mol Biol* 2005; 12: 737-8.
30. Sadoff BU, Heath-Pagliuso S, Castano IB, Zhu Y, Kieff FS, Christman MF. Isolation of mutants of Saccharomyces cerevisiae requiring DNA topoisomerase I. *Genetics* 1995; 141: 465-79.
31. Bennett CB, Lewis LK, Karthikeyan G*,* et al. Genes required for ionizing radiation resistance in yeast. *Nat Genet* 2001; 29: 426-34.
32. Keene JD, Tenenbaum SA. Eukaryotic mRNPs may represent posttranscriptional operons. Mol Cell 2002; 9: 1161-7.
33. Conaway JW, Conaway RC. Transcription elongation and human disease. *Annu Rev Biochem* 1999; 68: 301-19.

- - - -

THE COVER

Mistletoe and blackcap. Jürg Buess

Photo from Jürg Buess, Institute Hiscia, Arlesheim, Switzerland.
By courtesy of Dr. J. Kuehn

*Mistletoe growing on an apple tree, a bird, a female blackcap is picking a berry. The mistletoe (*Viscum album*, L) is a semi-parasite plant with evergreen leaves, stems and male and female flowers; the fruits are berries which ripen at the end of the autumn and are toxic for humans because they contain a viscotoxin. Mistleto grows on the branches of apple trees, poplars, birches, pines. Bird droppings disperse the seeds. Magic powers related to fertility and love have been attributed to mistletoe, as well as medicinal properties. In Europe, mistletoe is used in many pharmaceutical preparations.*

The blackcap (Sylvia atricapilla) *is a small bird, the size of a sparrow, with a characteristic cap, black in the male, and light brown in the female.*

- - - -

LA TAPA

Muérdago con curruca. Jürg Buess

Foto de Jürg Buess, Institute Hiscia, Arlesheim, Suiza.
Cortesía del Dr. J. Kuehn

Planta de muérdago que crece en un manzano, un pájaro, una curruca capirotada (*blackcap*) está picoteando un fruto. El muérdago, muérdago blanco, liga o visco (*Viscum album*, L) es una planta semi-parásita con hojas y tallos siempre verdes y flores masculinas y femeninas, el fruto son bayas que maduran al fin del otoño, venenosas para los humanos porque contienen una toxina (viscotoxina). El muérdago crece sobre las ramas de diversos árboles: manzanos, álamos, pinos. Se dispersa por las semillas que contienen las deyecciones de los pájaros. Al muérdago se le atribuyeron propiedades mágicas relacionadas con la fertilidad y el amor, también numerosas propiedades medicinales. En Europa se usa en muchas preparaciones farmacéuticas.

La curruca capirotada (*Sylvia atricapilla*, L) es un pájaro pequeño, del tamaño de un gorrión, con un característico capirote o gorra, negro en el macho (*blackcap*) y pardo o castaño rojizo en la hembra.

A menudo se confunde el muérdago con el acebo (*Ilex aquifolium,* L), "falso muérdago", usado en las decoraciones navideñas.