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Review

TIF-IA: An oncogenic target of pre-ribosomal RNA synthesis

Rui Jin [a], Wei Zhou [a,b,*]

[a] Department of Hematology and Medical Oncology, The Winship Cancer Institute of Emory University, Atlanta, GA 30322, USA
[b] Department of Pathology and Laboratory Medicine and Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

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ABSTRACT

Cancer cells devote the majority of their energy consumption to ribosome biogenesis, and pre-ribosomal RNA transcription accounts for 30–50% of all transcriptional activity. This aberrantly elevated biological activity is an attractive target for cancer therapeutic intervention if approaches can be developed to circumvent the development of side effects in normal cells. TIF-IA is a transcription factor that connects RNA polymerase I with the UBF/SL-1 complex to initiate the transcription of pre-ribosomal RNA. Its function is conserved in eukaryotes from yeast to mammals, and its activity is promoted by the phosphorylation of various oncogenic kinases in cancer cells. The depletion of TIF-IA induces cell death in lung cancer cells and mouse embryonic fibroblasts but not in several other normal tissue types evaluated in knock-out studies. Furthermore, the nuclear accumulation of TIF-IA under UTP down-regulated conditions requires the activity of LKB1 kinase, and LKB1-inactivated cancer cells are susceptible to cell death under such stress conditions. Therefore, TIF-IA may be a unique target to suppress ribosome biogenesis without significantly impacting the survival of normal tissues.

Contents

* Corresponding author at: Department of Hematology and Medical Oncology, Emory University School of Medicine, 1365 Clifton Road, NE, Suite C4084, Atlanta, GA 30322, USA.
E-mail address: wzhou2@emory.edu (W. Zhou).

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1. Introduction

Hyperactivation of ribosomal RNA (rRNA) transcription is an important molecular alteration in cancer cells. The nucleolus is the nuclear subdomain in which RNA polymerase I (RNA Pol I) transcription and the assembly of ribosomal subunits occur in eukaryotic cells. The abnormal nucleolus has been used as a marker for aggressive cancer for over 100 years [1], and more recent studies have linked dysregulated nucleolar morphology with the hyperactivation of rRNA transcription [2]. It has been estimated that approximately 80% of cancer cell energy consumption is used for rRNA biogenesis [3]. The synthesis of pre-rRNA, the first event in this process, is efficiently regulated mostly through reversible modification of RNA Pol I transcription factors [2]. 30–50% of RNA transcription in cancer cells is for pre-rRNA synthesis [4], and accelerated rRNA synthesis is one of the most important molecular alterations in cancer cells [5].

Pre-rRNA synthesis follows three essential steps: (i) the initial binding of the upstream binding factor (UBF) to the ribosomal DNA (rDNA) promoter leads to recruitment of the SL-1 (a.k.a. TIF-IB), (ii) the resultant UBF/SL-1 complex facilitates binding of an initiation-competent subpopulation of RNA Pol I, which is associated with TIF-IA, and (iii) TIF-IA mediates the interaction between RNA Pol I and SL-1, to form the pre-initiation complex at the rDNA promoter (Fig. 1). The regulation of rRNA synthesis involves many transcription factors and has been reviewed extensively [2,6,7]. This review focuses on TIF-IA, which is the rate-limiting factor in the initiation of pre-rRNA transcription and is activated by various oncogenic kinases in human cancers.

2. The TIF-IA gene: *RRN3*

2.1. The discovery of TIF-IA protein and the cloning of RRN3

In a human cell system, the combination of three well-purified factors, UBF, SL-1/TIF-IB, and RNA Pol I, was initially found to be sufficient to support the correct initiation of rDNA transcription *in vitro* with a suitable DNA template [8]. However, some suspected the involvement of other factors which may co-purify with these factors. This led to the discovery of TIF-IA protein, which is strongly associated with RNA Pol I [9]. The yeast homolog of *TIF-IA* gene is called *RRN3*, which was cloned from *Saccharomyces cerevisiae* in 1996 [10]. Human *TIF-IA* gene was cloned in 2000 [11,12]. Human TIF-IA protein is 21% identical and 43% similar to the yeast protein. The RNA Pol I transcription initiation complex in yeast is quite different from that in the mammalian system, and consists of four transcription factors, upstream activation factor (UAF), core factor (CF), TATA binding protein (TBP), and Rrn3p [4,13,14]. A transcription factor corresponding to UAF has not been found in the mammalian system [15]. However, human TIF-IA protein is capable of rescuing a yeast strain with a *RRN3* disruption, indicating TIF-IA-mediated regulation of ribosomal RNA synthesis is conserved among eukaryotes [11].

2.2. Analysis of RRN3 in human cancers

The original human TIF-IA gene, *RRN3*, locates on the short arm of chromosome 16 (16p13.11), and it contains 28 exons. An untranscribed pseudogene is found on chromosome 2p22.2, which may complicate the genetic analysis or manipulation of *RRN3* locus but should not affect expression based analysis such as RNAseq. However, there are two other duplicates of *RRN3* that are actively transcribed but unable to generate the TIF-IA protein [16]. The first duplicate copies the original gene to 16p11.2. It loses the last exon and also contains a 3517 base pair (bp) interstitial deletion which includes exons 11 and 12. This locus was duplicated again to 16p12.2. Again, this second duplication is also incomplete which results in the loss of the last three exons. These duplication events were found in the chimpanzee genome but not in the rhesus genome. Other vertebrate genomes and non-vertebrate organisms only have a single copy of the *RRN3* gene, indicating that these duplication events occurred after the human-chimp-rhesus split but before the human-chimp split [16]. The presence of these duplicates in human cells poses a challenge in the mutational analysis of the *RRN3* locus in human tumors (see below), and an obstacle in the manipulation of the *RRN3* locus, such as using CRISPR to introduce a specific mutation into the TIF-IA protein.

The *RRN3* locus is not frequently altered on a DNA level in human cancers, and it is unlikely to be a driver-gene for human cancers. Of all the studies available in the cBioportal database, only two of them report significant genetic alterations in *RRN3*. In a neuroendocrine prostate cancer study by Trento/Cornell/Broad, 17 (15.9%) out of 107 cases contain *RRN3* amplifications. In a breast cancer patient xenograft study, *RRN3* was found to be amplified in 6 (20.7%) out of 29 samples [17]. Of the 77 somatic *RRN3* mutations collected in all tumors analyzed by The Cancer Genome Atlas (TCGA), 8 (11%) are nonsense or frame-shift mutations. Interestingly, one third of these missense mutations occur either at codon 9 or codon 11. All 12 of the codon 11 mutations contain a proline to serine mutation, and most of the codon 9 mutations (12/13) contain arginine to cysteine mutation. The recurrent nature of these mutations suggested that such alterations may promote the function of TIF-IA as an oncogene [18]. However, a closer analysis indicated that the *RNN3* duplicate on 16p12.2 contains both alterations, and these missense mutations are not likely to be real genetic alterations of the original *RRN3* locus in human tumors.

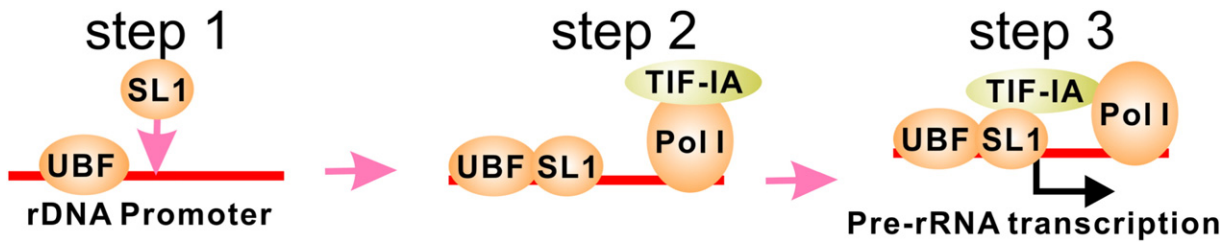

Fig. 1. Essential steps in the initiation of pre-rRNA synthesis.