Inhibition of Pol I Transcription a New Chance in the Fight Against Cancer

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Abstract

While new cancer treatments continue to improve patient outcomes, for some cancers there have been limited or no improvements for a long time. It is for these cases radically new approaches are required. Recent publications proposing ribosome biogenesis, in particular RNA polymerase I transcription, as a suitable target for cancer treatment has been gaining momentum. For example, we demonstrated that CX-5461, a specific RNA polymerase I transcription inhibitor, is effective in treating an aggressive subtype of acute myeloid leukemia, regardless of p53 status. Notably, CX-5461 reduces the leukemia initiating/stem cells, the cell population believed to be responsible for chemotherapy resistance and disease relapse in numerous cancers. Targeting ribosome biogenesis, once considered merely a "housekeeping process," is showing promise in a continuously growing list of cancers including lymphoma, prostate, and now acute myeloid leukemia. Evidence suggests the therapeutic efficacy of RNA polymerase I therapy in preclinical models is mediated through a variety of mechanisms including nucleolar stress activation of p53, DNA damage-like activation of ataxia-telangiectasia mutated/ataxia-telangiectasia and Rad3 related, and cellular differentiation. Overall, the available data suggests the potential for targeting ribosome biogenesis to be effective in a broad spectrum of cancers. The outcomes of 2 phase 1/2 trials of CX-5461 in hematological malignancies and breast cancer are eagerly awaited.

Keywords

ribosome biogenesis, RNA polymerase I transcription, CX-5461, cancer therapy, acute myeloid leukemia, leukemia initiating cells, p53 pathway, DNA damage-like pathway, differentiation, cell cycle defect

Abbreviations

AML, acute myeloid leukemia; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3 related; LIC, leukemia initiating/stem cells; MLL, mixed-lineage leukemia; Pol I, RNA polymerase I; rDNA, ribosomal DNA.

Commentary

A growing number of studies have placed ribosome biogenesis and its cellular location, the nucleolus, into the spotlight as a novel target for cancer therapy. This is remarkable as, for a significant period of time, ribosome biogenesis was considered merely a "housekeeping process" implicated purely in defining the translational capacity of cells. The recent and ever increasing body of work, primarily the result of the "omics" era, is collectively placing the regulation of ribosome biogenesis and the nucleolus as an essential hub for cellular homeostasis and whose dysregulation has broad consequences for the cell. Indeed, there are now studies demonstrating that dysregulation of ribosome biogenesis in diseases, such as cancer and ribosomopathies, impacts on the nucleolus to control the activity of tumor suppressors, oncogenes, global genome stability, and cell fate decisions.[1-4]

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The first step in the synthesis of new ribosomes is the transcription of the ribosomal RNA genes (rRNA) by RNA polymerase I (Pol I). This process is highly upregulated in many malignant cells compared to their nonmalignant counterparts through the action of oncogenes such as MYC and the release from inhibition by tumor suppressors such as the retinoblastoma protein or p53. Intriguingly, although it remained unrecognized for many years, it appears that many highly effective chemotherapeutics for cancer strongly perturb ribosome biogenesis at the step of rDNA transcription and/or ribosomal RNA processing; examples include platinum-based chemotherapeutics such as Oxaliplatin or Cisplatin and antimetabolites such as 5-Fluorouracil.[5] This suggests that at least part of their efficacy might be mediated through inhibition of ribosome biogenesis rather than the traditional view that their cytotoxicity is mediated solely by damaging DNA. Although these chemotherapeutic drugs in many cases significantly reduced disease burden and prolonged patient survival, their major drawback is the severe toxicity caused by their concomitant ability to induce genome-wide DNA damage in surrounding normal tissue. In the longer term, this frequently results in the development of secondary neoplasms such as therapy-related acute myeloid leukemia (AML) and myelodysplasia. For example, the incidence rate after conventional therapy to develop these secondary malignancies ranges between 0.8% and 6.3%.[6] These observations led to the development of small molecules that selectively inhibit Pol I transcription including CX-5461 (Senhwa Biosciences, San Diego, CA) to target malignancies with reduced toxicity and improve efficacy compared to conventional chemotherapies that target multiple processes.[7,8]

One cancer whose core cytotoxic therapy has not substantially changed over the last 30 years is AML. In 2016 alone, 19 950 new AML cases and 10 430 disease-related deaths were reported in the United States.[9] Acute myeloid leukemia is a disease of the elderly with an incidence of 1.3 annual cases per 100 000 under the age of 65 years increasing to 12.2 cases per 100 000 in individuals over the age of 65 years. The 5-year survival rate for patients with *de novo* AML lies around 27%.[9,10] Therapy-related AML, however, has a more unfavorable prognosis due to the accumulation of mutations such as those in the tumor suppressor p53 induced through chemotherapy and/or radiation.

These statistics reveal the urgent need for the development of new efficacious therapies preferentially without severe side effects. A promising novel therapeutic agent falling in this category is the selective Pol I transcription inhibitor CX-5461. We tested CX-5461 on a particularly aggressive subtype of AML, mixed-lineage leukemia (MLL)–driven AML, which fails to response to standard of care treatment. Our studies found that maximum-tolerated doses of CX-5461 outperformed standard of care therapy (cytarabine/doxorubicin) in 2 different murine models of MLL-AML.[4] Remarkably, in this study, the therapeutic response differed from that observed in B-cell lymphoma where CX-5461 mediated its response in a p53-dependent manner. CX-5461 therapy significantly
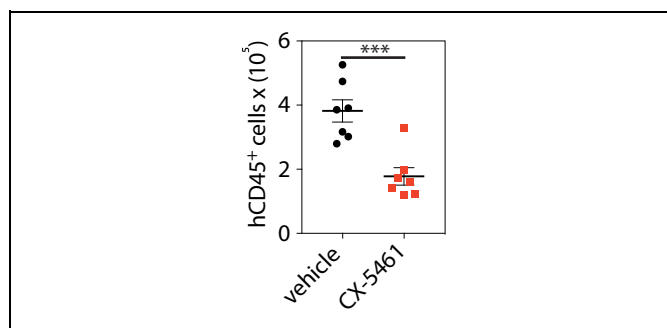

Figure 1. CX-5461 has significant therapeutic potential in primary human AML. CX-5461 therapy (6 doses, 40 mg/kg) reduces the number of primary human CD45$^+$ cells in the bone marrow of engrafted NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/Sz) mice (***$P =$ .0006; n = 7). Graph represents mean (SD). Unpaired 2-tailed Student t test. AML indicates acute myeloid leukemia.

prolonged overall survival in both p53 wild-type and p53 null AML. The finding that CX-5461 treats p53 null AML has significant clinical relevance as the frequency of p53 mutation increases over the course of conventional chemotherapies treatment and at disease relapse. CX-5461 not only treated syngeneic models of murine AML but also significantly reduced tumor burden in patient-derived xenograft of primary human AML (Figure 1). These studies suggest that pharmacological inhibition of Pol I transcription could be utilized as a novel therapy option for patients with refractory AML.

Intriguingly, inhibition of Pol I transcription induces a heterogeneous AML tumor cell response. Induction of p53-dependent apoptosis and defects in G1/S transition were observed, as well as, the activation of a p53-independent DNA damage-like response mediated via ATM/ATR activation altering G2/M cell cycle progression and induction of differentiation[1,4] (Figure 2). Our study demonstrated that CX-5461 p53-independent activation of ATM/ATR signaling occurred in the absence of global DNA damage. Mechanistically, we showed that CX-5461 induces an unusual chromatin structure in which transcriptionally competent relaxed rDNA repeats are devoid of transcribing Pol I, leading to noncanonical activation of ATM signaling selectively within the rDNA in the nucleoli. Since CX-5461 does not induce global DNA damage, the risk of the development of secondary therapy–related cancers would be reduced compared to other chemotherapy treatments, for example, cytarabine and anthracycline-based regimes that cause the genome-wide accumulation of DNA damage.

In addition to apoptosis and cell cycle defects, Pol I inhibition by CX-5461 triggered myeloid differentiation in leukemic blasts *in vivo* which is likely to contribute to the drug's therapeutic efficacy. This observation is consistent with an increasing number of studies describing a direct link between the rate of Pol I transcription/ribosome biogenesis and differentiation. In particular, it appears that ongoing Pol I transcription is important for stem cell maintenance, and its downregulation is associated with the loss of stemness and induction of
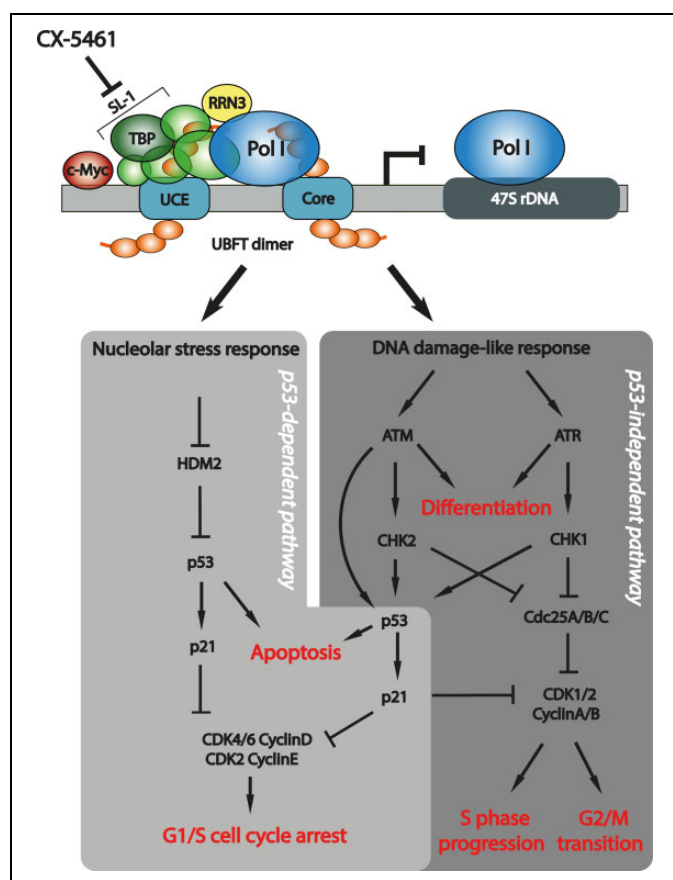
Figure 2. Inhibition of RNA polymerase I transcription activates p53-dependent and independent pathways. Schematic overview of downstream signaling pathways activated by pharmacological inhibition of Pol I transcription. CX-5461 inhibits Pol I transcription at the stage of initiation activating 2 main pathways: a canonical nucleolar stress response leading to accumulation of p53 and a DNA damage-like response activating ATM/ATR signaling. Both pathways induce different cellular responses including apoptosis, G1/S, and G2/M cell cycle defects and/or differentiation. ATM indicates ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3 related; CDK, cyclin-dependent kinases; CHK, checkpoint kinases; HDM2, human double minute 2; Pol I, RNA polymerase I; rDNA, ribosomal DNA; SL-1, selectivity factor 1; TBP, TATA-binding protein; UBFT, upstream binding factor; UCE, upstream control element.

differentiation in the stem/progenitor cells.[2,11,12] Interestingly, recent research demonstrated that the abundance of a number of Pol I-specific transcription factors, including UBFT and SL-1, decreases upon differentiation that correlates with downregulation of Pol I transcription.[13] On the other hand, downregulation of Pol I transcription by targeted knockdown of another Pol I-specific transcription factor RRN3 Transcription initiation factor-1A induces myeloid differentiation in human AML cell lines and mouse hematopoietic stem cells.[11] Other evidence supporting a role of Pol I transcription in stemness comes from the discovery that numerous factors involved in ribosome biogenesis are enriched in stem/progenitor cells; for example, TAF1B, TCOF1, and fibrillarin and a number of the "canonical" pluripotency factors such as Oct3-4/POU5F1,

Sox2, Nanog, and KLF4 directly associated with the rDNA in mouse embryonic stem cells.[14]

Our observation that Pol I transcription regulates stem cell maintenance and differentiation has significant implications for the clinic. Specifically, inhibition of Pol I transcription reduced the clonogenic potential of malignant cells and most importantly reduced the frequency of leukemia initiating/stem cell (LIC). The LIC is the cell population believed to be responsible for chemotherapy resistance and disease relapse in numerous cancers. Many chemotherapies ultimately fail because while they reduce the bulk of the tumor they do not eradicate the LIC. A drug's ability to target LICs will have a profound impact on clinical efficacy and is absolutely essential in achieving long-term remission and disease-free survival. This is not only relevant to leukemia but also a growing number of cancers, that is, breast and colorectal cancers are postulated to have a cancer initiating/stem cell population.

In conclusion, our work demonstrates that CX-5461 treats aggressive hematologic malignancies. Most strikingly, we show that targeting rDNA transcription effectively prolongs survival by reducing the leukemia-initiating potential and suppresses the clonogenic capacity of the LICs population in primary human and murine models of AML.

Cumulatively, these findings and favorable toxicology studies have led to the initiation of 2 clinical trials, a phase I in Australia for patients with advanced hematologic malignancies (Australian New Zealand Clinical Trials Registry, #12613001061729) and a phase I/II in Canada (NCT02719977) for the treatment of solid tumors.

Finally, we note that despite CX-5461 providing significant improvements in the therapeutic window in our cancer models compared to conventional therapies, the disease often relapses. Thus, future studies must focus on the development of effective combination therapies. So far, promising results have been achieved by dual inhibition of Pol I transcription and ribosome function in B-cell lymphoma[15] and models of prostate cancer.[16] It is important to note that we are developing second-generation Pol I inhibitors focusing on improving its drug like qualities and penetrance of the blood–brain barrier to open up new treatment options for patients with metastatic disease.

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