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EXPERT OPINION

1. Introduction
2. Selective inhibitors of Pol I transcription as cancer therapeutics
3. Expert opinion

Targeting RNA polymerase I transcription and the nucleolus for cancer therapy

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The nucleoli are the site of the production of ribosomes, the protein synthetic apparatus of the cell. The presence of enlarged nucleoli, reflecting increased ribosomal gene transcription, has long been used by pathologists as an indicator of aggressive tumors. However, over the last 10 years a growing body of evidence has revealed that the nucleolus contains a dynamic cohort of over 4500 proteins, the majority of which have no function in ribosome production. The activity of some of these proteins is modulated by their regulated sequestration and release from the nucleolus. In particular, the nucleolus plays a central role in sensing cellular stress to modulate the abundance of the critical tumor suppressor protein p53. The finding that p53 activity is dysregulated in up to 50% of all human cancers highlights the importance of the nucleolar stress response in limiting malignant transformation. The development of drugs to selectively inhibit transcription of the ribosomal RNA genes in the nucleolus has paved the way for a new therapeutic approach to hijack nucleolar stress to selectively and non-genotoxically activate p53 in tumor cells. Here, we describe the potential application of this exciting new class of drugs for the treatment of human cancer.

Keywords: 5-FU, actinomycin D, CX-5461, MDM2, nucleolar stress, nucleolar surveillance, p53, ribosomal RNA, RNA polymerase I transcription

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1. Introduction

Transcription of the ribosomal RNA genes (rDNA) gives rise to the 28S, 5.8S and 18S ribosomal RNAs (rRNAs), the catalytic backbone of the ribosomes, which translate all cellular proteins [1]. As such, rDNA transcription is a critical determinant of the proliferative growth rate of cells. rRNA synthesis takes place in specialized subnuclear domains termed nucleoli that are formed around actively transcribed rDNA. Perhaps not surprisingly, elevated rDNA transcription by the dedicated RNA polymerase I (Pol I) enzyme is consistently elevated in tumor cells [1]; while enlarged and often multiple nucleoli, a consequence of elevated rDNA transcription and ribosome biogenesis, have been used by pathologist for over 100 years as a marker of cellular transformation [2].

Remarkably, recent spatiotemporal proteomic studies have demonstrated that in excess of 4500 proteins are associated with nucleoli, of which only 30% have functions relating to ribosome biogenesis [3]. The activity of many of these non-ribosome-associated nucleolar proteins is controlled by their regulated sequestration or release from the nucleolus, giving rise to the concept that the nucleolus controls many cellular functions in addition to ribosome biogenesis [4-6]. Perhaps, the best-described extra ribosomal function associated with the nucleolus is the control of the critical tumor suppressor protein TP53 (p53). Specifically, it has been shown that perturbations that acutely disrupt ribosome biogenesis and/or nucleolar

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integrity lead to the induction of a nucleolar stress response in which ribosomal proteins that are no longer involved in ribosome biogenesis bind to the ubiquitin ligase MDM2 (HDM2 in humans) and prevent it from ubiquitinating p53 [4-6]. This leads to the accumulation of p53 and downstream effects such as senescence, cell cycle arrest, autophagy and apoptosis depending on the cell type (**Figure 1**).

2. Selective inhibitors of Pol I transcription as cancer therapeutics

The above observations raise the possibility that small molecule inhibitors that selectively block rDNA transcription might be used to reactivate the nucleolar stress response in tumor cells as a non-genotoxic mechanism to induce p53 and its tumor suppressor functions [7]. Indeed, there have been numerous clinically approved cytotoxic drugs whose therapeutic affect is associated with disruption of ribosome biogenesis including actinomycin D (dactinomycin), cisplatin, ironotican/topotican, mitomycin C, 5-fluorouracil and derivatives of rapamycin (**Table 1**) [8-10]. However, none of these drugs are selective enough for Pol I transcription to allow definitive conclusions as to how much of their therapeutic effect is mediated via Pol I [9,10].

An important advance in this area has been the development of the first small molecule inhibitors, which preferentially target Pol I transcription; for example, CX-5461 (Cylene Pharmaceuticals), which specifically prevents the Pol I transcription initiation factor, SL-1, binding to the rDNA promoter [10,11]. Building on these data, further studies using CX-5461 and genetic approaches have provided unequivocal evidence that accelerated rDNA transcription and nucleolar integrity are necessary for oncogenic activity in hematologic tumors driven by the c-MYC oncogene [12]. Furthermore, it was demonstrated that Pol I transcription could be targeted *in vivo* to selectively activate p53-dependent apoptosis in cancer cells, effectively treating tumors in both genetically engineered and xenograft models of lymphoma and leukemia [12]. Intriguingly, the induction of p53-mediated apoptotic death of the tumor cells was rapid, occurring within hours of treatment as a result of nucleolar stress and was independent of changes in total ribosome levels or protein translation [12]. This later observation is critical as it demonstrates that Pol I transcription and nucleolar integrity are acutely required for the survival of certain tumor cells, independent of protein synthesis and cell proliferation. Based on these results and favorably toxicity profile, CX-5461 is currently undergoing a Phase I/II clinical trial in patients with hematologic malignancies at the Peter MacCallum Cancer Centre, Australia.

3. Expert opinion

Perhaps, the major challenge for this new class of cancer therapeutics lies in defining how and when 'Pol I transcription therapy' will be used in the clinic. The fact that many existing cytotoxic drugs also indirectly target Pol I transcription and/or ribosome biogenesis suggests that drugs designed to interfere with this process might be most effectively used as broad-spectrum chemo-therapeutics that reactivate p53 in actively proliferating cells [9]. If this is the case, it is reasonable to ask what advantages would such a drug have over many currently used chemotherapeutics or γ-irradiation?

We believe the most striking advantage Pol I transcription therapies may have over these standard treatments is that they will be far less genotoxic (DNA damaging) to the non-tumor cell population. For example, *in vivo* concentrations of CX-5461 that activate p53 and induce apoptosis in malignant B cells do not activate p53 in normal B cells, or other cells of the hematopoietic system examined, and do not elicit any evidence of DNA damage in any of these cell types [12]. This is despite the drug being equally potent in inhibiting Pol I transcription in normal and tumor cells. Thus it appears normal cells have a much higher threshold for activation of nucleolar stress and stabilization of p53 in response to Pol I transcription inhibition, than tumor cells. In contrast, cytotoxic drugs or γ-irradiation generally induce DNA damage and p53 in both the tumor cell population and the surrounding normal cells. Consequently, we expect CX-5461 and other inhibitors of Pol I transcription to exhibit far better therapeutic windows and be associated with a significantly reduced incidence of secondary cancers compared to standard DNA damaging agents.

The concept of non-genotoxically activating p53 to treat cancer is not new. Considerable effort has gone into developing these compounds in the past, however moderate potency and limited therapeutic window has dogged their transition into the clinic. Perhaps the most clinically advanced is RG7112 (Hoffman-La Roche), a small-molecule inhibitor that functions by inhibiting the interaction between MDM2 and p53 leading to p53 stabilization and accumulation [13]. We believe that reactivation of p53 through nucleolar stress will have distinct advantages over stabilization of p53 through MDM2 antagonists that target MDM2 in both normal and tumor cells. This stabilization of p53 in non-tumor cells most likely accounts for the often dose-limiting myelosuppressive effects (including thrombocytopenia, anemia, and neutropenia) of RG7112 [14]. In contrast, rDNA transcription inhibitors activate p53 via a nucleolar stress response, which is suppressed in normal cells and highly sensitized in tumor cells. Thus we predict that drugs like CX-5461 will have a significantly greater therapeutic window than MDM2 antagonists.

Importantly, the analysis of a large panel of diverse solid and hematologic cancer cell types revealed that p53 wild-type hematologic tumors are the most sensitive to inhibition of Pol I transcription, suggesting that response to Pol I transcription therapy may be more selective than previously thought [11]. Why hematologic tumors should be more sensitive than solid tumors is not clear, although it is generally recognized that they undergo apoptosis in response to

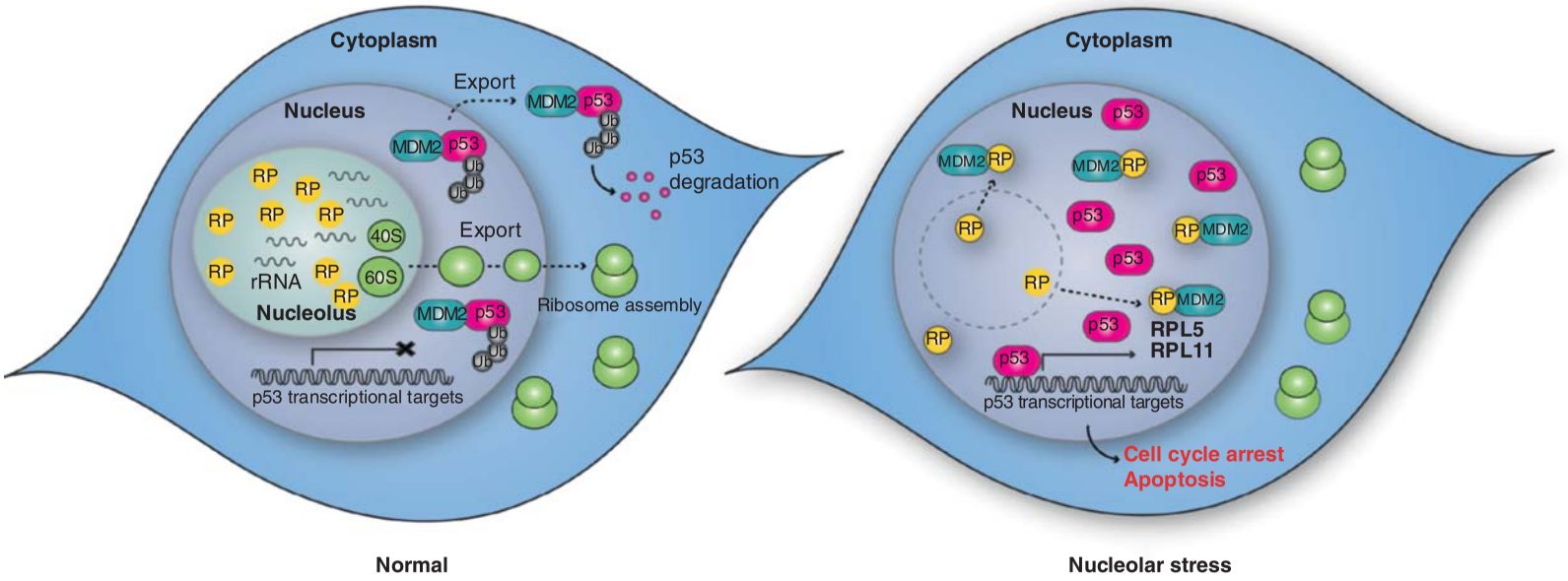

Figure 1. Schematic representation of the 'canonical' nucleolar stress response. Under normal growth conditions (left panel), the RPs are assembled with rRNAs into ribosomal subunits in the nucleolus and transported to the cytoplasm to form functional ribosomes. p53 activity is maintained at low levels by MDM2, via two mechanisms: i) MDM2 ubiquitinates p53 to promote its degradation; ii) the binding of MDM2 to p53 abrogates its interaction with Pol II transcription machinery. However in response to agents that induce nuclear stress like CX-5461 (right panel), the nucleolus is disrupted leading to the release of free RPs to the nucleoplasm where they bind MDM2 and prevent its interaction with p53 leading to increased p53 stability. The activation of p53 induces cell cycle arrest, apoptosis and/or senescence. Note additional molecules and mechanisms have been implicated in the nuclear stress response but are not shown for simplicity [4-6].

Table 1. Clinically approved drugs whose therapeutic effect is associated with disruption of ribosome biogenesis.

Drug	Mechanism of action	Impact on ribosome biogenesis	Impact on nucleolus	Cancer type(s)
5-Fluorouracil	Thymidylate synthase, incorporates into 47S pre-rRNA	Impairs late rRNA processing	No effect	Colon, esophageal, gastric, rectum, breast, biliary tract, stomach, head and neck, cervical, pancreas, renal cell, and carcinoid cancer
Actinomycin D	Intercalates into GC-rich duplex DNA	Inhibits Pol I transcription at low nano-molar concentrations	Nucleolar disintegration	Wilms tumour and Ewing sarcoma
Camptothecin (Topotecan and Irinotecan)	Inhibits Topoisomerase I	Modulates early rRNA processing	Nucleolar disintegration	Ovarian, lung, colon and cervical cancer
Cisplatin	DNA cross-linking via alkylating DNA bases	Inhibition of Pol I transcription	Nucleolar disintegration	Testicular, bladder, lung, esophagus, stomach, ovarian sarcoma, lymphoma
CX-3543	Disrupts nucleolin/rDNA G-quadruplex complexes	Selective inhibition of Pol I transcription (elongation)	Redistribution of nucleolin, no effect on fibrillarin	Phase I clinical trial Carcinoid and neuroendocrine tumors
CX-5461	Inhibits SL-1 pre-initiation complex formation at the rDNA	Selective inhibition of Pol I transcription (initiation)	Nucleolar disintegration	Phase I clinical trial AML, multiple myeloma, lymphoma
Doxorubicin	Intercalates into DNA and inhibits Topoisomerase II	Inhibition of Pol I transcription	Nucleolar disintegration	Bladder, breast, stomach, lung, ovarian and thyroid cancer, leukemia, Hodgkin's lymphoma, myeloma
Homoharringtonine	Translation inhibitor, prevents elongation	Impairs late rRNA processing	No effect	Chronic myelogenous leukemia (CML)
Mitomycin C	Interstrand DNA cross-linking via alkylating 5-CpG-3 guanosine	Inhibition of Pol I transcription	Nucleolar disintegration	Adenocarcinoma stomach, pancreas, anal, bladder, breast, cervical, colorectal, head, neck, and non-small cell lung cancer
Mitoxantrone	Topoisomerase II inhibitor and intercalates into DNA	Inhibition of Pol I transcription	Nucleolar disintegration	Breast, prostate, liver cancer, myeloid leukemia, non-Hodgkin's lymphoma
Oxaliplatin	DNA cross-linking via alkylating DNA bases	Inhibition of Pol I transcription	Nucleolar disintegration	Esophagus, stomach cancer, colorectal carcinoma
Temsirolimus everolimus	mTOR inhibitors	Inhibition of Pol I transcription	No effect	Renal cell carcinoma, subependymal giant cell astrocytoma (SEGA), progressive neuroendocrine tumors of pancreatic origin (PNET), subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TS)

reactivation of p53 more readily than wild-type p53 solid tumors. However, we believe that the oncogene MYC may also play a critical role in determining the sensitivity of hematologic tumor cells, and potentially some solid tumors to Pol I inhibition. MYC is the most potent modulator of rRNA synthesis and ribosome biogenesis described to date, transcriptionally regulating a cohort of factors required for Pol I transcription and also directly activating the rDNA genes [1]. MYC is also frequently upregulated in hematologic tumors even when it is not the driving oncogene.

In the Eμ-MYC model of lymphoma, pre-malignant Eμ-MYC lymphoma cells demonstrated the same high sensitivity to Pol I inhibition and apoptotic response as did the fully malignant Eμ-MYC lymphoma cells, despite exhibiting few genetic lesions in addition to elevated MYC expression [12]. Thus, MYC overexpression alone, independent of transformation, can be sufficient to sensitize cells to RNA Pol I inhibition. Consistent with these data, we have found that overexpression of MYC but not other oncogenes such as RAS is sufficient to increase sensitivity to Pol I inhibition in human fibroblasts (Hannan and Pearson unpublished data). Moreover, pharmacogenomic analysis of a large panel of ovarian cancer cell lines demonstrated that MYC overexpression correlated significantly with high sensitivity to Pol I inhibition (Hannan and Pearson, unpublished data). The enhanced response of tumor cells with elevated MYC following inhibition of Pol I transcription may be a function of the robust upregulation of ribosome biogenesis that occurs in response to MYC – sensitizing cells to the induction of the nucleolar stress pathway. The MYC-induced upregulation of rRNA synthesis also requires a stoichiometric elevation in the levels of ribosomal proteins in the nucleolus. Selective inhibition of rRNA synthesis in these cells would result in excessive accumulation of free ribosomal proteins and subsequent activation of the nucleolar stress pathway and p53.

One potential limitation of Pol I transcription inhibitors is that their sensitivity may be restricted to the subset of tumor cells with elevated ribosome biogenesis and high proliferative capacity. However, the current data showing that cells respond acutely to Pol I inhibition independent of downstream affects on ribosome biogenesis and proliferative cell growth indicate that cells can sense changes in Pol I transcription directly [12]. Thus the sensitivity of cancer cells to Pol I transcription therapy may not be linked directly to their proliferative capacity and thus may also provide a novel therapeutic option for more indolent malignancies such as certain multiple myelomas and mantle cell lymphomas.

In summary, the non-genotoxic reactivation of p53 through nuclear stress promises to be a new avenue for treatment of certain cancers affording considerable less toxicity than current approaches. Optimization of treatment protocols and the identification of rational combination therapies with this new class of drugs is a largely untapped field and has the potential to improve the efficacy and range of these drugs even further. The outcomes of the current ongoing Phase I/II trial of CX-5461 at the Peter MacCallum Cancer Centre, Australia are eagerly awaited.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hannan KM, Sanij E, Rothblum LI, et al. Dysregulation of RNA polymerase I transcription during disease. Biochim Biophys Acta 2013;342-60
2. Derenzini M, Montanaro L, Trere D. What the nucleolus says to a tumour pathologist. Histopathology 2009;753-62

• **A comprehensive overview of the relationship between nuclolar size and cancer pathology.**

3. Andersen JS, Lyon CE, Fox AH, et al. Directed proteomic analysis of the human nucleolus. Curr Biol 2002;1-11

•• **A landmark paper demonstrating the large protein complexity and dynamic nature of the nucleolus supporting the view that the nucleolus may perform additional functions beyond its known role in ribosome subunit biogenesis.**

4. Olson MO. Sensing cellular stress: another new function for the nucleolus? Sci STKE 2004;2004(224):pe10
5. Boisvert FM, van Koningsbruggen S, Navascues J, et al. The multifunctional nucleolus. Nat Rev Mol Cell Biol 2007;574-85
6. Boulon S, Westman BJ, Hutten S, et al. The nucleolus under stress. Mol Cell 2010;216-27
7. Ruggero D. Revisiting the nucleolus: from marker to dynamic integrator of cancer signaling. Sci Signal 2012;5(241):pe38
8. Burger K, Muhl B, Harasim T, et al. Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. J Biol Chem 2010;12416-25

•• **The first systematic analysis demonstrating that that inhibition of ribosome biogenesis by chemotherapeutic drugs may potentially contribute to their therapeutic efficacy.**

9. Drygin D, Rice WG, Grummt I. The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. Annu Rev Pharmacol Toxicol 2010;131-56

• **A comprehensive overview of Pol I transcription as a target for cancer therapy.**

10. Bywater MJ, Pearson RB, McArthur GA, et al. Dysregulation of the basal RNA polymerase transcription apparatus in cancer. Nat Rev Cancer 2013;299-314
11. Drygin D, Lin A, Bliesath J, et al. Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. Cancer Res 2011;1418-30

•• **This paper describes the development of the first selective inhibitor of RNA Pol I.**

12. Bywater MJ, Poortinga G, Sanij E, et al. Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. Cancer Cell 2012;51-65

•• **The first definative evidence that cancer cells can be dependent on high rates of RNA Pol I transcription, and that this dependency can be targeted by a small-molecule inhibitor that selectively inhibits RNA Pol I transcription.**

13. Tovar C, Graves B, Packman K, et al. MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. Cancer Res 2013;2587-97
14. Ray-Coquard I, Blay YJ, Italiano A, et al. Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. Lancet Oncol 2012;1133-40

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