Fall 10-22-2023

Significance of Targeting RNA Polymerase I in Intrahepatic Cholangiocarcinoma

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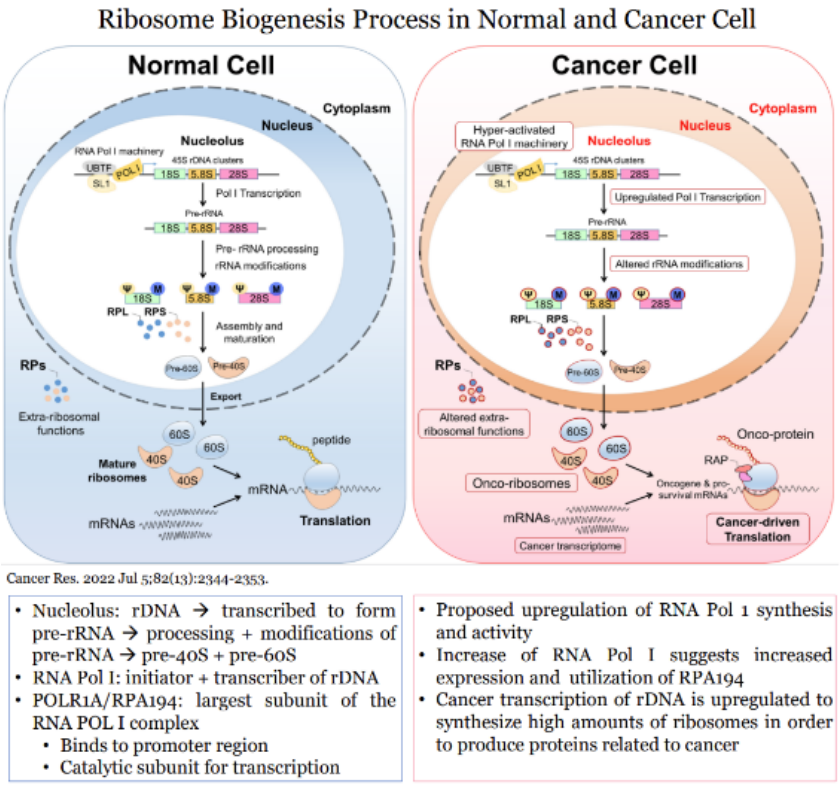
Introduction

Intrahepatic cholangiocarcinoma (IHCC) is a much-overlooked cancer with a mortality rate that has increased throughout recent years, as stated by the American Cancer Society [1]. In the United States alone, there are an estimated 8,000 adults being diagnosed with IHCC every year, with a five-year survival rate of 9% [2]. Chemotherapy options for the treatment of IHCC include systemic chemotherapy such as gemcitabine, capecitabine, and oxaliplatin. These medications carry a wide array of adverse factors that may warrant discontinuation due to the detriment to the well-being of the patient. Additionally, a broad field of therapy that may also be used, even throughout many other types of cancers, is aimed to arm the immune system, such as targeted therapy as well as immunotherapy. Even with the consideration of these options, we cannot say that our issue has been solved. If the cancer is diagnosed at an early stage and treated adequately, the five-year survival rate is still at 24% [2], which will still result in the mortality of majority of the individuals with IHCC. Therefore, the discovery of new potential molecular targets is required which could be used in rationale designing of the prevention and treatment strategies against advanced IHCC.

The ribosome biogenesis process is dysregulated in most cancer cells because of the high demand of protein synthesis. However, the role of ribosome biogenesis components was the least studied in cancer settings. From our extensive research in various systems, we found that POLR1A (RPA194), a catalytic subunit of RNA polymerase I, is significantly overexpressed ($P<0.0001$) in intrahepatic cholangiocarcinoma tissues compared to normal tissues. In our research on hepatocellular carcinoma as well as pancreatic ductal adenocarcinoma, we have evaluated the anti-cancer efficacy of specific pharmacological inhibitors of POLR1A by using an in vitro as well as an in vivo mouse model system. We observed that targeting POLR1A induces apoptosis and suppresses the growth of several human liver cancer cell lines. Further progressing our research in testing the inhibitor on cholangiocarcinoma cell lines would prove to give a great insight on the impact of ribosomal biogenesis in the progression of IHCC. Overall, these results suggest that further research on the role and inhibition of POLR1A could be a novel potential molecular target and prognostic biomarker for cholangiocarcinoma.



Cancer Res. 2022 Jul 5;82(13):2344-2353.

- Nucleolus: rDNA → transcribed to form pre-rRNA → processing + modifications of pre-rRNA → pre-40S + pre-60S
- RNA Pol I: initiator + transcriber of rDNA
- POLR1A/RPA194: largest subunit of the RNA POL I complex
  - Binds to promoter region
  - Catalytic subunit for transcription

- Proposed upregulation of RNA Pol 1 synthesis and activity
- Increase of RNA Pol I suggests increased expression and utilization of RPA194
- Cancer transcription of rDNA is upregulated to synthesize high amounts of ribosomes in order to produce proteins related to cancer

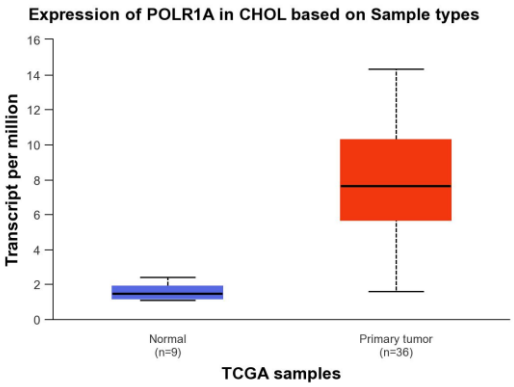
A breakdown of ribosomal biogenesis in normal versus cancer cells

Methods

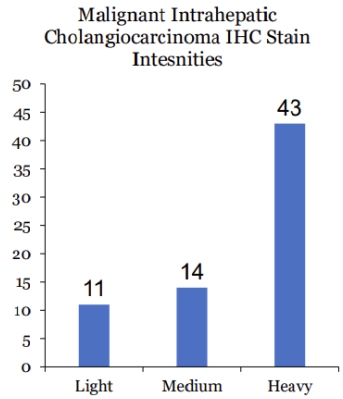
- <u>In silico Analysis:</u> We used various bioinformatic tools (UALCAN, TIMER, Expression Atlas, and NIH GDC) to determine the expression patterns of various components of ribosome biogenesis in normal and tumor liver tissues.
    - To make note of, protein and genetic bioinformation is scantly available when researching for genes associated with ribosomal biogenesis in cholangiocarcinoma. This should further interest us to gather more information about this disease.
- <u>Immunohistochemistry:</u> To investigate the expression of RPA194 in liver hepatocellular carcinoma and cholangiocarcinoma tissues as well as normal liver tissues.

Results

Ribosome Biogenesis Components are Overexpressed in Intrahepatic Cholangiocarcinoma Tissue Compared to Normal Tissue

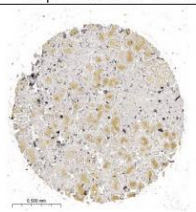
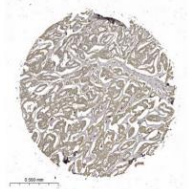
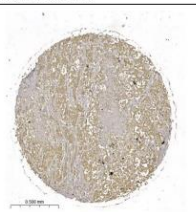
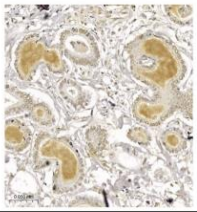
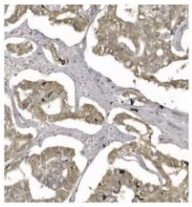
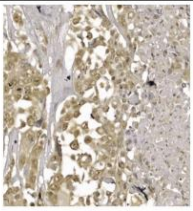


There is a significantly higher expression of POLR1A ($p<0.0001$) in primary cancer tissue compared to normal tissue. (UALCAN)



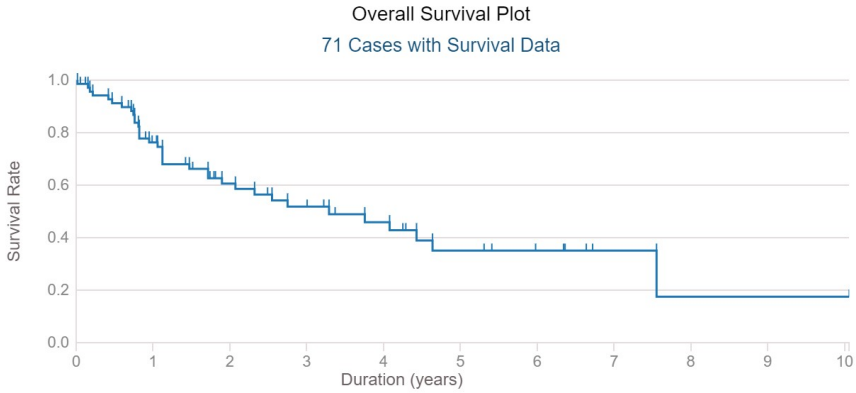
Within an IHCC tissue array slide of 100 samples, 68 samples have stained from light to heavy.

Expression of RPA 194 in Malignant Intrahepatic Cholangiocarcinoma

7x			
40x			
Grade	1	2	3
Stage	II	IVa	IVa

Immunohistochemistry results showing localized staining within intrahepatic cholangiocytes, with staining of the nuclei. Mouse antibody targeting human POLR1A sc-48385 at a dilution of 1:50.

High POLR1A Expression is Linked to Overall Poor Prognosis in Liver and Cholagniocarcinoma



Kaplan-Meier survival curve for IHCC within a span of 10 years. An approximately 50% chance of survival within a timeframe of three to four years in a patient sample with high expression of POLR1A.

Discussion

Our findings on the impact of POLR1A as well as its inhibition on both pancreatic and liver cancer in our other investigations showed strong suggestion that targeting this mechanism of ribosomal biogenesis may yield to be fruitful. Further research was done to assess bioinformatic data on POLR1A's role in the progression of intrahepatic cholangiocarcinoma. Compared to the normal sample, there is a significantly increased expression of POLR1A ($p<0.0001$) within intrahepatic cholangiocarcinoma compared to normal tissue. At least 68 tissue samples were detected with high POLR1A expression, especially within the nuclear region in immunohistochemistry. An issue that has come up during in-silico analysis is the scarcity of bioinformatics and proteomics on bile duct cancers regarding genes associated with ribosomal biogenesis. Some inference towards liver cancer may be the closest data that is available.

These findings may further warrant additional work on determining the role of POLR1A as well as its inhibition on the impact of cancer progression. POLR1A inhibition in other cancers such as hepatocellular carcinoma and pancreatic ducal adenocarcinoma done in our ongoing investigations have yielded positive results, thus further sanctioning its therapeutic effect in intrahepatic as well as possible extrahepatic cholangiocarcinoma. Procedures such as western blotting, immunofluorescence as well as preclinical mouse models may prove to provide a great insight into this disease.

References

1. Patel N, Benipal B. Incidence of Cholangiocarcinoma in the USA from 2001 to 2015: A US Cancer Statistics Analysis of 50 States. Cureus. 2019 Jan 25;11(1):e3962. doi: 10.7759/cureus.3962. PMID: 30956914; PMCID: PMC6436669.
2. American Society of Clinical Oncology Cancer.Net Editorial Board. Bile Duct Cancer (Cholangiocarcinoma): Statistics ASCO 2023. https://www.cancer.net/cancer-types/bile-duct-cancer-cholangiocarcinoma/statistics