

Progetto di dottorato in BMC , XL Ciclo, Università Roma Tre

«New putative roles of RTEL-1 helicase in premature chromatid separation (PCS), spindle assembly checkpoint (SAC) and cell cycle progression»

Dottoranda: Elisa Amore

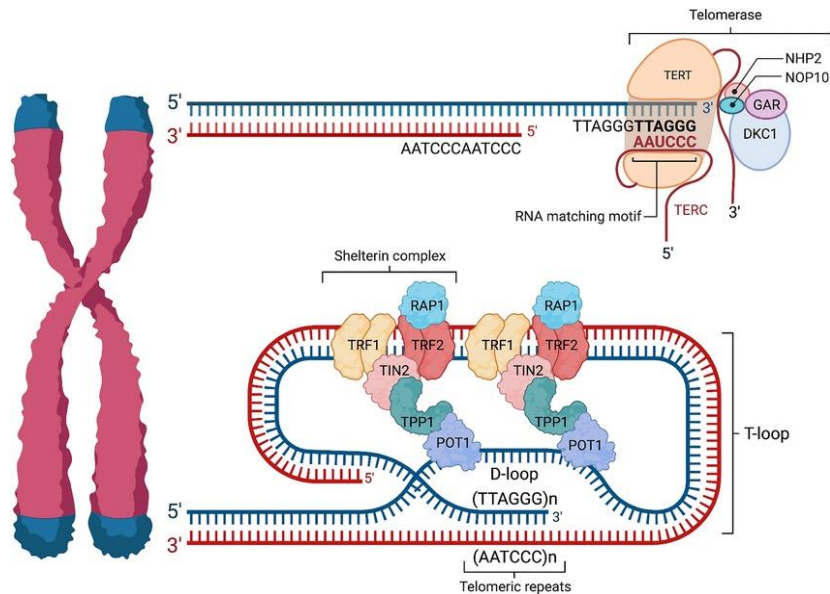
Roma, 14-03-25

Tutor: Antonio Antoccia

Co-tutor: Francesco Berardinelli

OUTLINE

- 1) Introduction and preliminary data
- 2) Aim of the project
- 3) Experimental plan: a) 1° Year
 - b) 2° Year
 - c) 3° Year



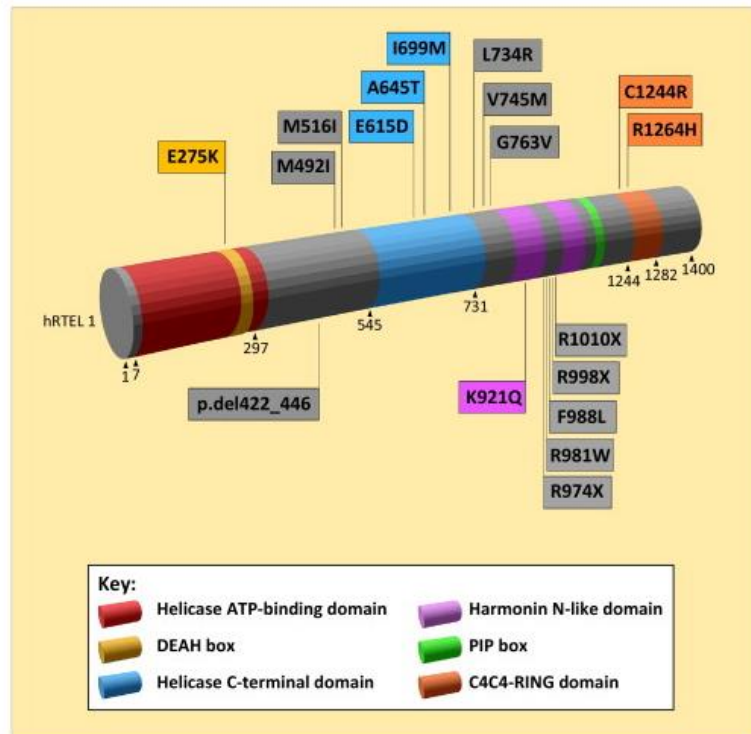
Muoio, Daniela & Laspata, Natalie & Fouquerel, Elise. (2022).
 Functions of ADP-ribose transferases in the maintenance of
 telomere integrity. *Cellular and Molecular Life Sciences*. 79.

10.1007/s00018-022-04235-z

Telomere: structure and function

- ▶ Telomeres are nucleoprotein structures located at the ends of linear chromosomes
- ▶ Maintain genomic stability by protecting chromosomes from DNA repair mechanisms
- ▶ T-loop, G-quadruplex, Shelterin complex

RTEL-1 structure



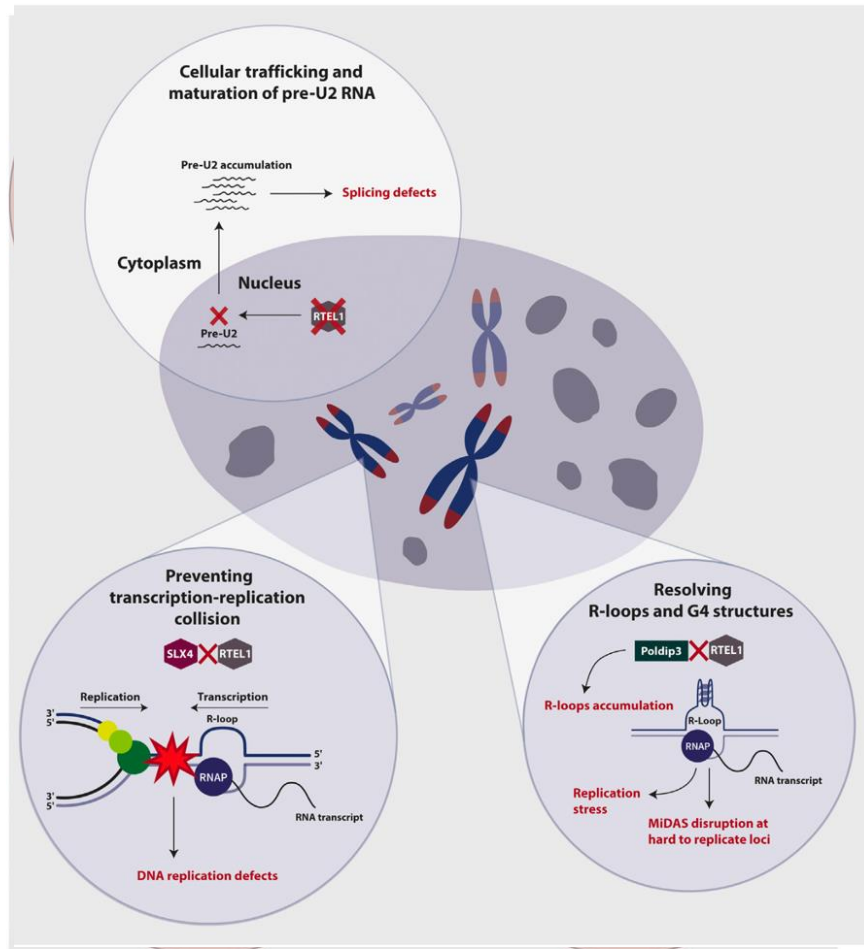
Jean-Baptiste Vannier, Grzegorz Sarek, Simon J. Boulton, *RTEL1: functions of a disease-associated helicase*, Trends in Cell Biology, Volume 24, Issue 7, 2014

- ▶ RTEL-1 (regulator of telomere elongation helicase 1)
- ▶ 20q13.3
- ▶ Fe-S helicase
- ▶ SF2 superfamily (RTEL-1, XPD, FANCI, **DDX11**)

1. Warsaw breakage syndrome (WBS) - rare cohesinopathy

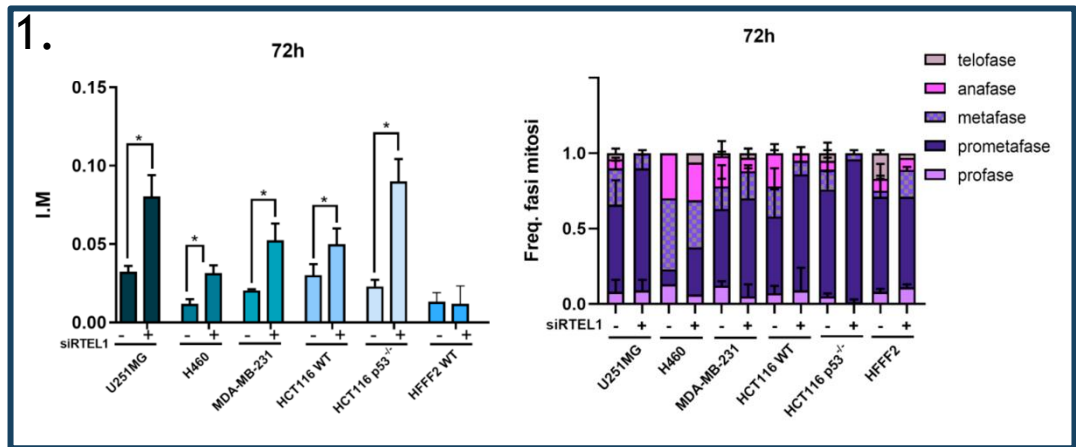
2. Structural similarities with RTEL-1 gene

RTEL-1: function

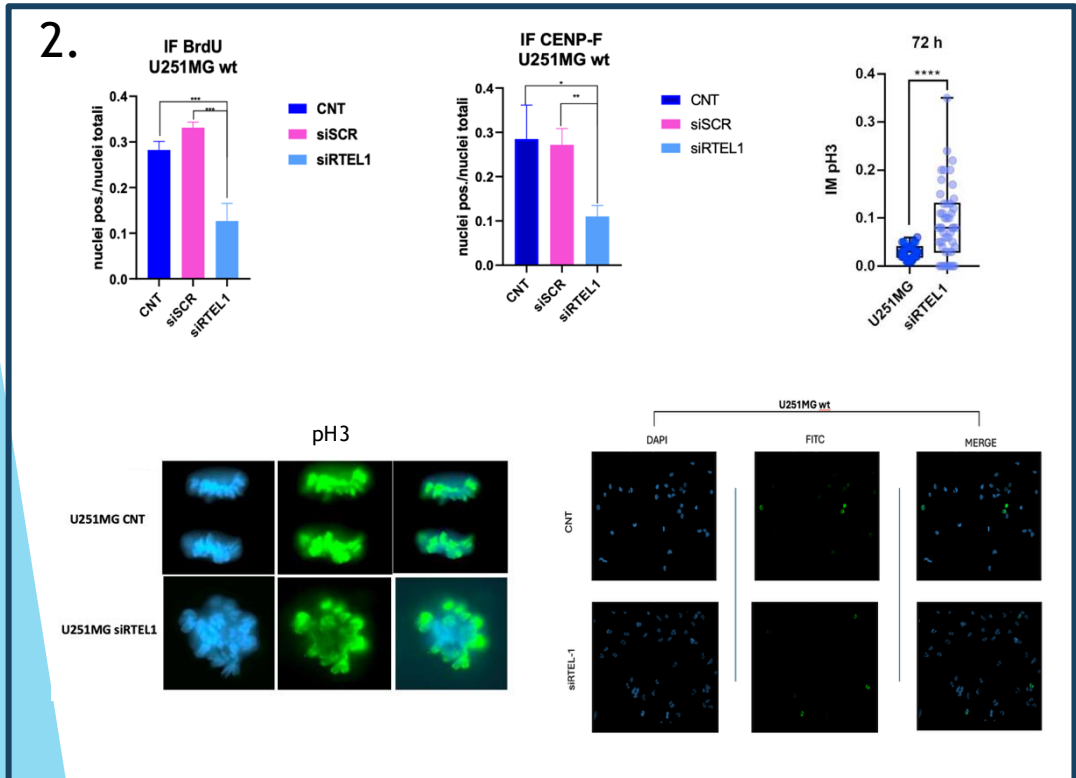


- ▶ Maintain telomere integrity and genomic stability
- ▶ Both its downregulation and its upregulation based on the cellular context can lead to tumor formation
- ▶ Lack of RTEL1 leads to transcription–replication collisions (TRC) accumulation, replication stress (RS), and disruption of mitotic DNA synthesis (MiDAS) at loci difficult to replicate
- ▶ Transcriptional silencing and control of DNA methylation and trimethylation of H3K27
- ▶ Hoyerlaal-Hreidarsson syndrome (HHS)

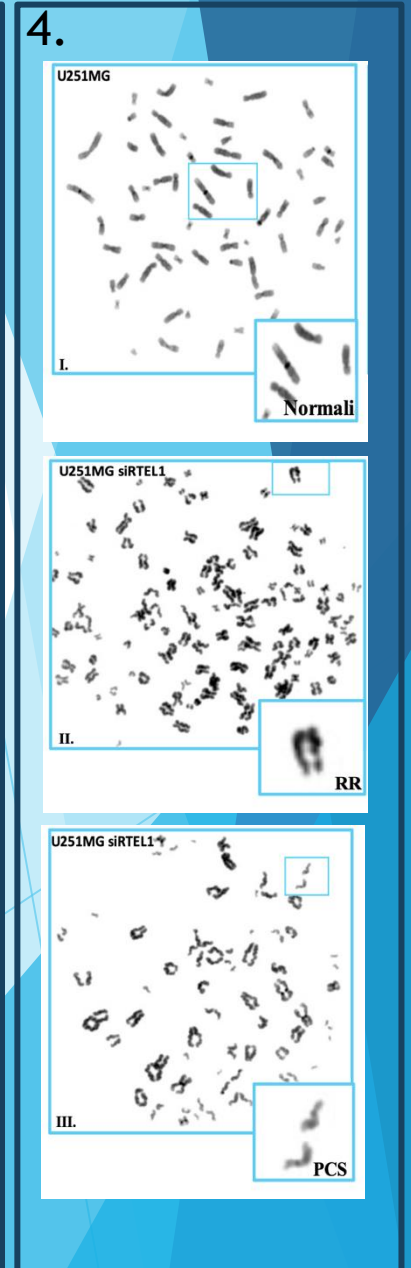
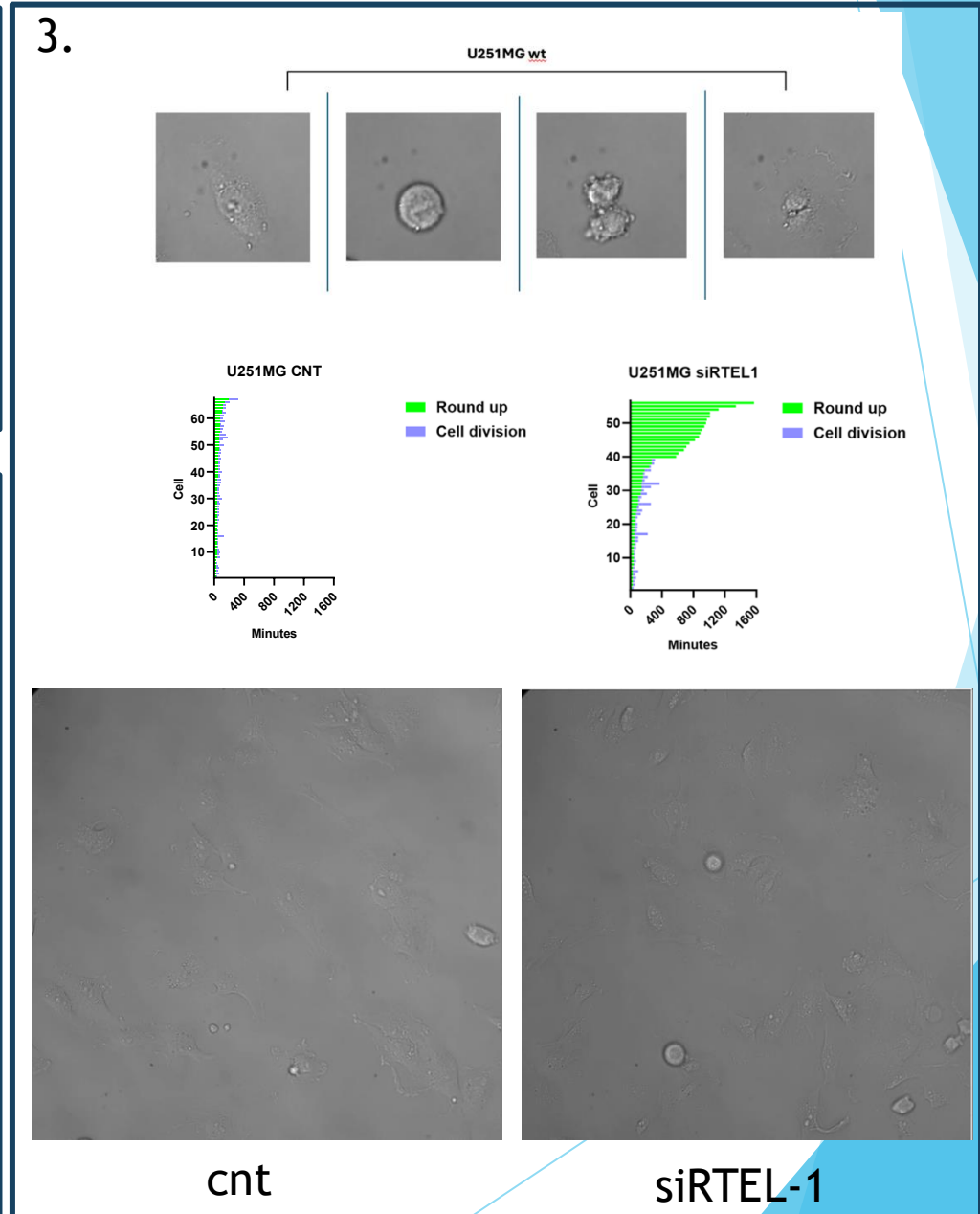
siRTEL-1 causes accumulation in prometaphase



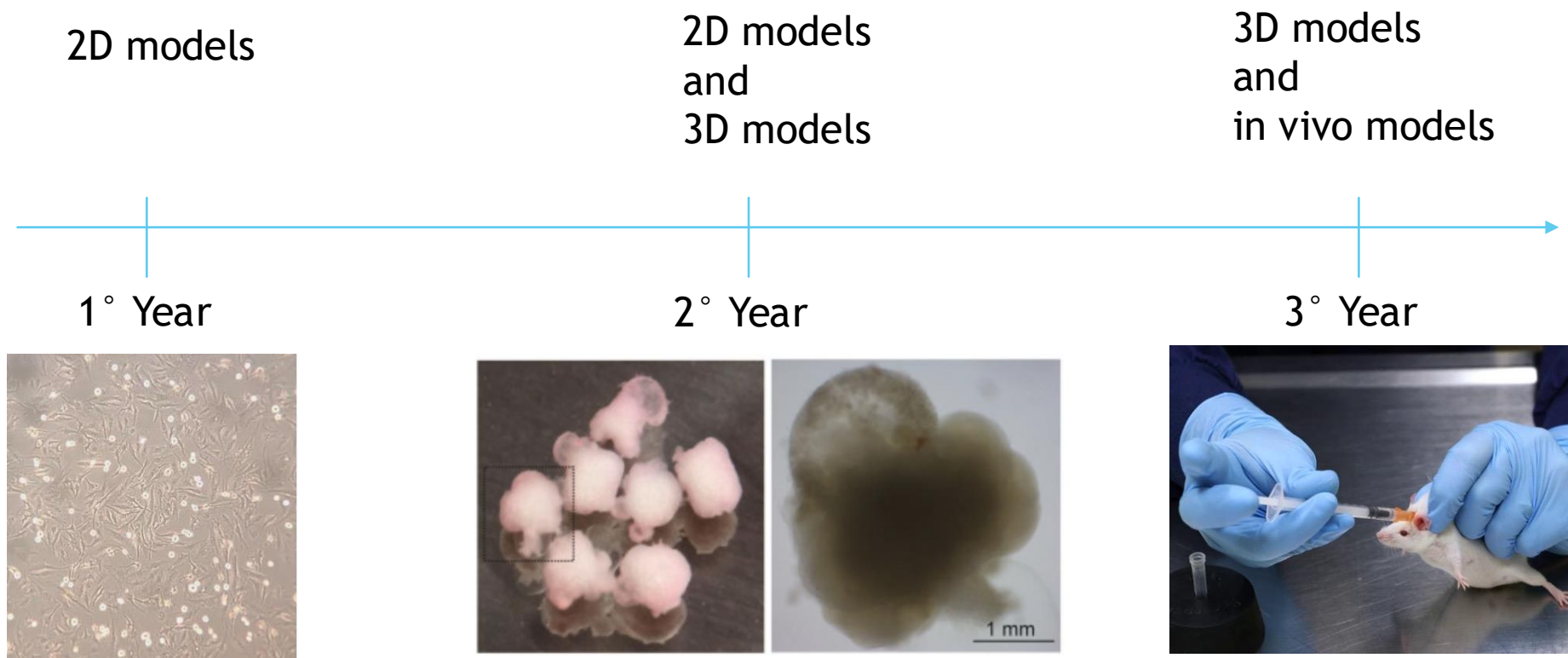
Confirm of cell cycle deregulation in immunofluorescence



Validation of M-phase arrest in time-lapse



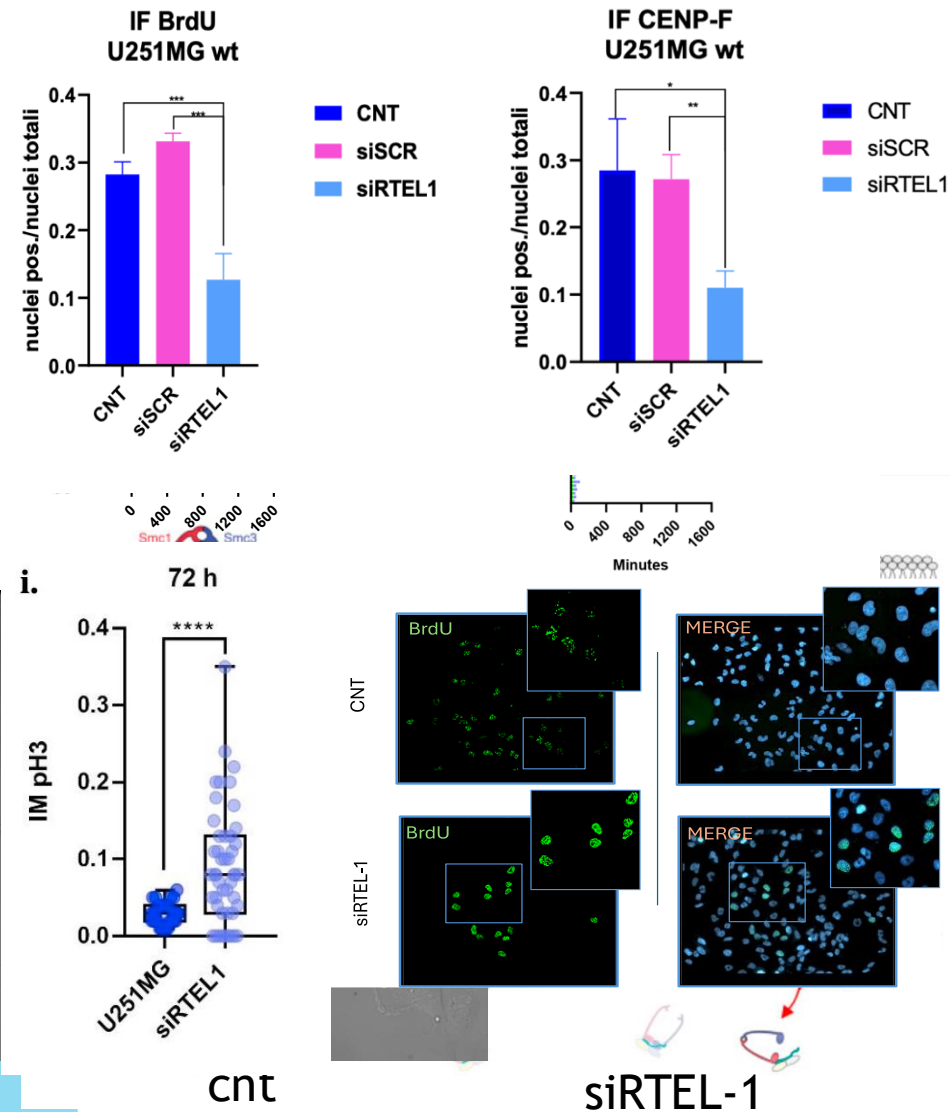
- ▶ New putative roles of RTEL-1 helicase in premature chromatid separation (PCS), spindle assembly checkpoint (SAC) and mitotic progression



Mariappan A, Goranci-Buzhala G, Ricci-Vitiani L, Pallini R, Gopalakrishnan J. Trends and challenges in modeling glioma using 3D human brain organoids. *Cell Death Differ.* 2021 Jan;28(1):15-23. doi:10.1038/s41418-020-00679-7. Epub 2020 Dec 1. PMID: 33262470; PMCID: PMC7707134.

JoVE Science Education Database. Lab Animal Research. Compound Administration I. JoVE, Cambridge, MA, (2023).

Preliminary data

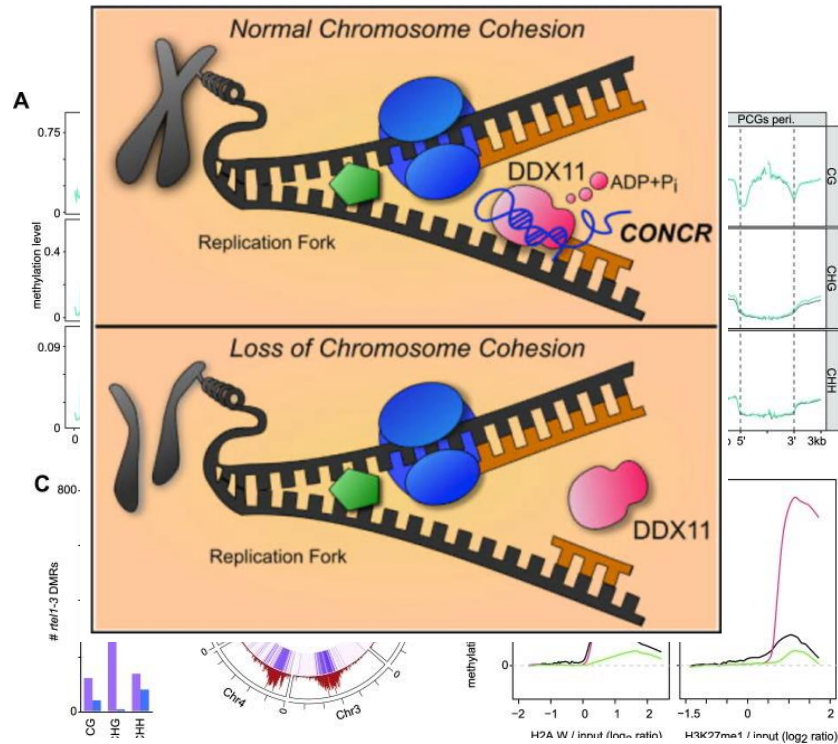


1° Year aims

1. Verify PCS in HCT116 p53^{-/-} (FISH)
2. Compare HCT116 p53^{-/-} and wt regarding the effects due to transient silencing of RTEL-1 by siRNA
3. Immunofluorescence experiments will also be carried out to determine what happens in the different phases of the cell cycle
4. TIME-LAPSE analysis in DIC
5. Study the mechanism responsible for PCS:
 - a) WB (Smc1 and Smc3, scc1 and scc3)
 - b) RT-PCR and WB (SGO1/2)
 - c) co-immunoprecipitation experiments and/or proximity ligation assay (PLA) (CENP-A and SGO1/2 and between SGO1/2 and cohesin)
 - d) Immuno- FISH (phosphorylates cohesin) and co-immunofluorescence (CENP-A and phosphorylates cohesin)

1. Study the mechanism responsible for PCS
2. Characterize the role of RTEL-1 in cell cycle progression

2° Year aims



Marchese FP, Grossi E, Marín-Béjar O, Bharti SK, Raimondi I, González J, Martínez-Herrera DJ, Athie A, Amadoz A, Brosh RM Jr, Huarte M. A Long Noncoding RNA Regulates Sister Chromatid Cohesion. *Mol Cell*. 2016 Aug 4;63(3):397-407. doi: 10.1016/j.molcel.2016.06.031. Epub 2016 Jul 28. PMID: 27477908; PMCID: PMC5893147. Modified by Amore Elisavira for silencing and epigenome stability. *Nucleic Acids Res*. 2023 Sep 8;51(16):8463-8479. doi: 10.1093/nar/gkad610. PMID: 37471026; PMCID: PMC10484728

1. Analyze if there is a modulation of expression also in some transcribed involved in the regulation of the cell cycle progression, in the spindle assembly checkpoint (SAC) and in the PCS using data from Gene Expression Omnibus (GEO) database.

(Olivier M, Hesketh A, Pouch-Pélissier MN, Pélissier T et al. RTEL1 is required for silencing and epigenome stability. *Nucleic Acids Res* 2023 Sep 8;51(16):8463-8479. PMID: 37471026)

(Long E, Patel H, Golden A, Antony M, Yin J, Funderburk K, Feng J, Song L, Hoskins JW, Amundadottir LT, Hung RJ, Amos CI, Shi J, Rothman N, Lan Q; International Lung Cancer Consortium; Choi J. High-throughput characterization of functional variants highlights heterogeneity and polygenicity underlying lung cancer susceptibility. *Am J Hum Genet*. 2024 Jul 11;111(7):1405-1419. doi: 10.1016/j.ajhg.2024.05.021. Epub 2024 Jun 20. PMID: 38906146.

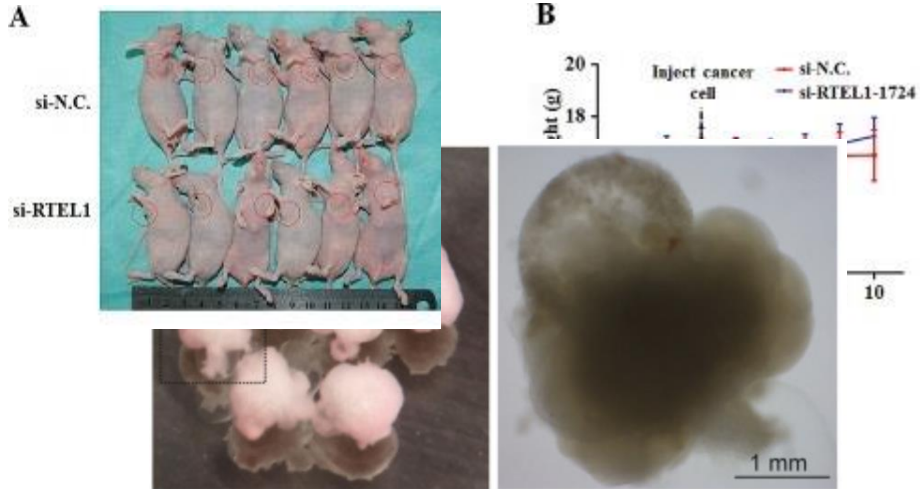
2. Transcriptomic analysis using Illumina HiSeq 4000 on U251MG wt cells and RTEL-1 silenced

1. Analyze data from GEO on transcribed involved in SAC, PCS and cell cycle progression
2. Transcriptomic analysis on U251MG
3. Analyze and validate the results obtained through transcriptomic

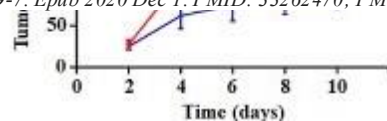
3° Year aims

1. Produce a line of U251MG RTEL1 -/- (CRISPR-CAS9)
2. Verify whether the results obtained from transcriptomics regarding the specific targets downstream of the pathway in which RTEL-1 operates have also been modified in 3D models of brain cancer organoids.
3. See whether the tumor blocks its growth following the lack of expression of RTEL-1 (or genes downstream of its pathway) in vivo in a mouse model
4. Immunohistochemistry experiments (Ki67, cyclins, PCNA, Caspase-3 and 9)

1. Produce a line of U251MG RTEL1 -/-
2. Create a brain tumor organoid
3. Produce a xenograft tumor in vivo
4. Immunohistochemistry



Mariappan A, Goranci-Buzhala G, Ricci-Vitiani L, Pallini R, Gopalakrishnan J. Trends and challenges in modeling glioma using 3D human brain organoids. *Cell Death Differ.* 2021 Jan;28(1):15-23. doi:10.1038/s41418-020-00679-7. Epub 2020 Dec 1. PMID: 33262470; PMCID: PMC7707134.



Wang G, Ren X, Li J, Cui R, Zhao X, Sui F, Liu J, Chen P, Yang Q, Ji M, Hou P, Gao K, Qu Y. High expression of RTEL1 predicates worse progression in gliomas and promotes tumorigenesis through JNK/ELK1 cascade. *BMC Cancer.* 2024 Mar 26;24(1):385. doi:10.1186/s12885-024-12134-8. PMID: 38532312; PMCID: PMC10967111. modified by Amore Elisa

Project objectives

- ▶ Confirmation of preliminary data obtained in my thesis project on HCT116 wt and p53 -/-
- ▶ Study the mechanism responsible for PCS
- ▶ Transcriptomic analysis on U251MG wt (cell cycle progression, SAC and in the PCS)
- ▶ Verify if there is an arrest in tumor growth or its regression which will allow us to understand whether targeting RTEL-1 or its effectors downstream of the pathway can be in the future proposed as targets for therapeutic purposes.

THANKS FOR YOUR ATTENTION