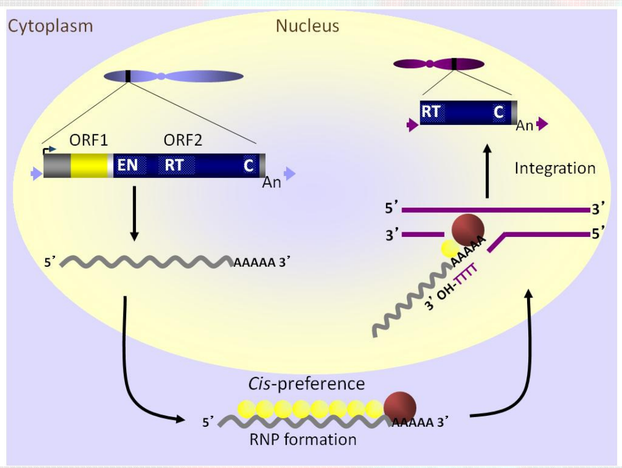
The impact of mutations and pharmacological drugs on L1 retrotransposition efficiency

Amir Zakirov, Sofia Laskina, Elizaveta Drapkina, Anastasia Kalinina, Sergei Starikov, Guilllem Rosselló, Elkhov Grigor, Carles Albàs Boix, Anna Ganicheva, Maria Fedoseeva, Anna Potapova, Natasha Sukharicheva, Thomas Widmann, José Luis García Pérez

Introduction

Only 5% of the human is made of coding sequences. Surprisingly, almost half of our genome is made of repetitive DNA. Among them, there are some DNA stretches that can relocate/move and insert into the genome: Transposable Elements. Most Transposable Element copies in our genome have been inactivated during evolution: however, the mobilization of Long Interspersed Non-autonomous Element-1 (LINE-1) continues to change the human genome.



L1 retrotransposition

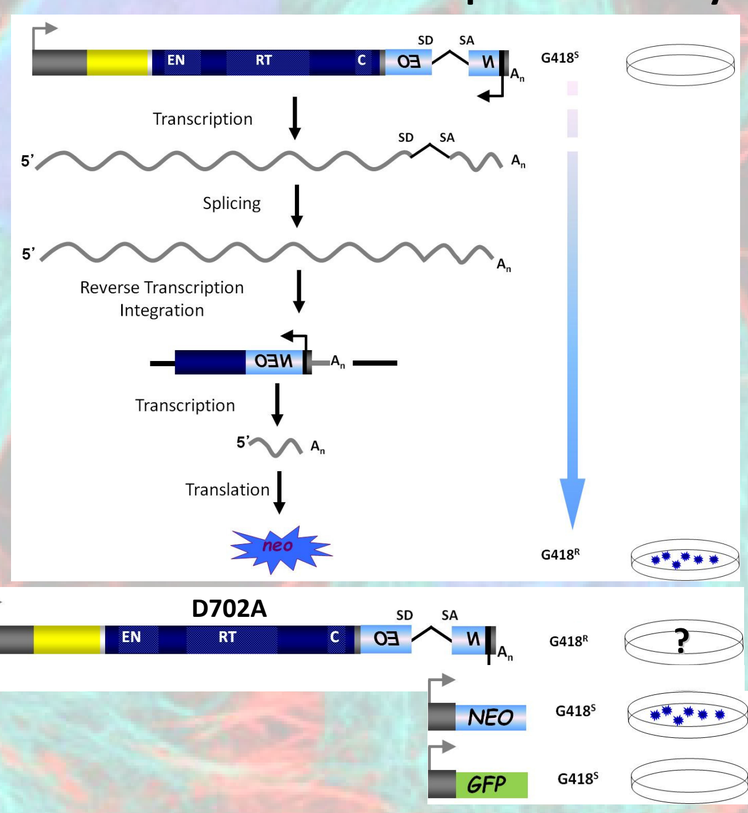
Therefore it is crucial to study the impact of ongoing LINE-1 retrotransposition. Their activity can alter the genome in a myriad of ways, and can sporadically lead to a number of diseases. However, it is also clear that their activity can generate new gene regulatory sequences.

Aim: the study of the impact of mutations and pharmacological drugs on the retrotransposition frequency of an active human LINE-1.

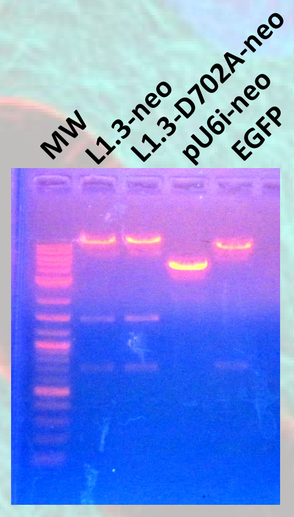
Methods

Extraction and purification of plasmid DNA using a MidiPrep kit (Thermo Scientific)
HeLa cells transfection with vectors, containing LINE-1 and a reporter gene (neomycin)
Fixation of cells with 2% HCHO in PBS and staining with crystal violet

Rationale of the retrotransposition assay

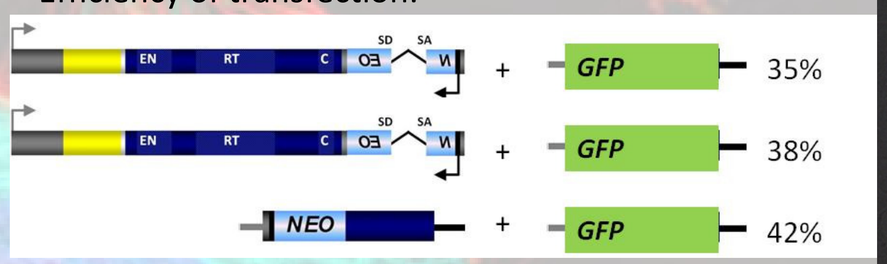


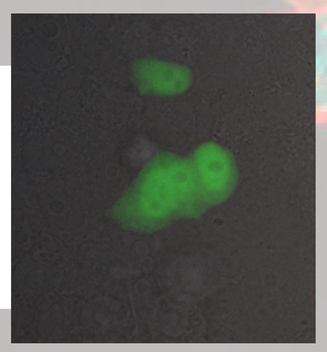
DNA preparation



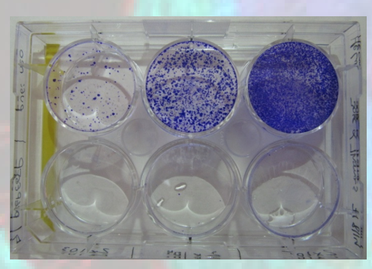
Results

Efficiency of transfection:



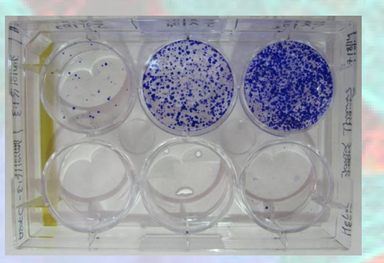


A) Retrotransposition assay



L1.3 wt

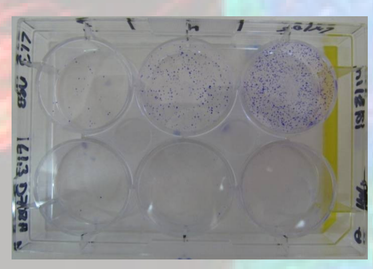
L1.3 mut D702A



pu6i neo

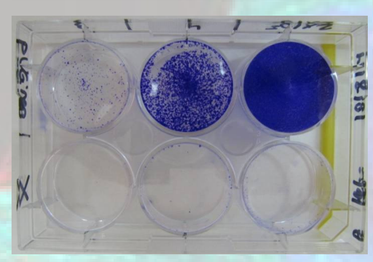
GFP

10 days G418 selection



L1.3 wt

L1.3 mut D702A

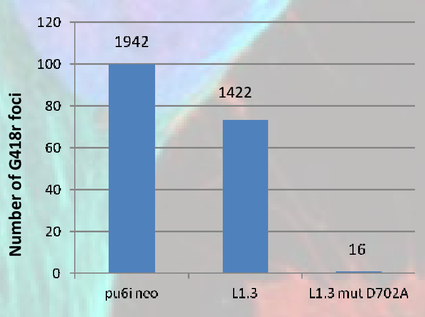


pu6i neo

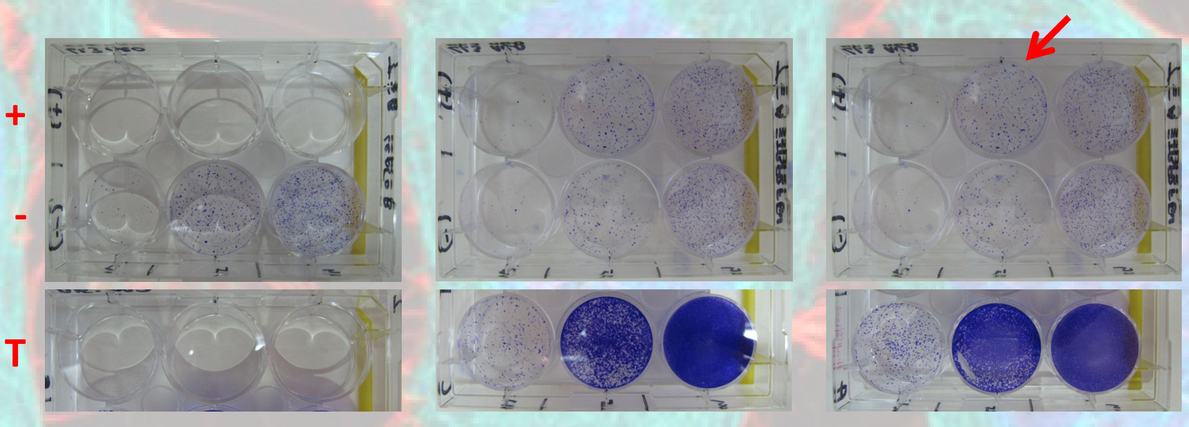
GFP

6 days G418 selection

Assay could not be completed



B) Effect of pharmacological drugs on retrotransposition frequency



Leptomycin B: Toxic for HeLa cells

Acyclovir: Non-toxic, no effect on retrotransposition

Ibuprofen: Non-toxic, small increase of retrotransposition rate

+ : L1.3 with drug; **- :** L1.3 alone; **T:** Toxicity assay (Drug alone)

Conclusions

- Leptomycine B was significantly toxic for HeLa cells in concentration of 50 nM (24h). Thus, it's influence on retrotransposition could not be estimated under our experimental conditions.
- Acyclovir didn't have any noticeable impact on the efficiency of retrotransposition.
- Ibuprofen caused a slight increase in the level of retrotransposition.

Future directions

- Mutate other conserved domains on LINE-1 encoded proteins and test their effect on retrotransposition.
- Conduct further assays with different ibuprofen concentrations and a modified experimental design. Test additional commonly used drugs (acetaminophen, pain killers, etc).