Gene therapy project

30/10/23

1 slide/group

Title
General Idea
Aim

Gene therapy project

Theme I: Aging
Group A: Bernardi, Ilie, Colonnelli, Bastianelli
Charcot marie tooth – pmp22
Group B: Hazrati, Bartolini, Glaudo, Montrone, Pourali
Spastic paraplegias 3a – atl1

Theme II: Cancer
Group C: Belvedere, Jeong, Majaliwa, Virgilio
Retinoblastoma – rb1
Group D: Santacroce, Pace, Serra, Fanelli, Duarte
Hepatic cancer – RACGAP1

Gene therapy project

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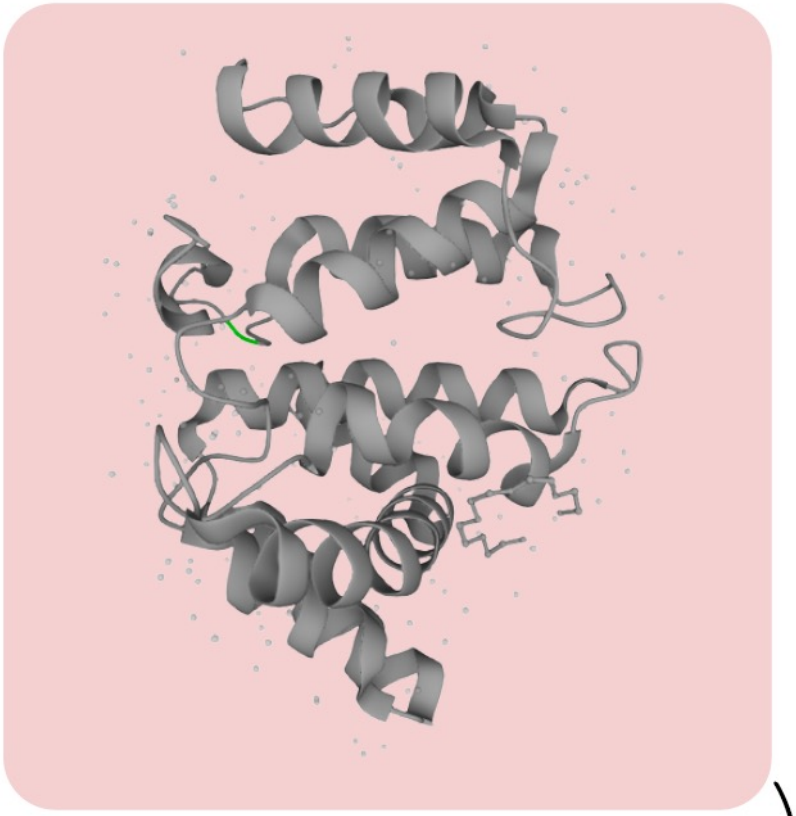
Cancer

Group D: Santacroce, Pace, Serra, Fanelli, Duarte

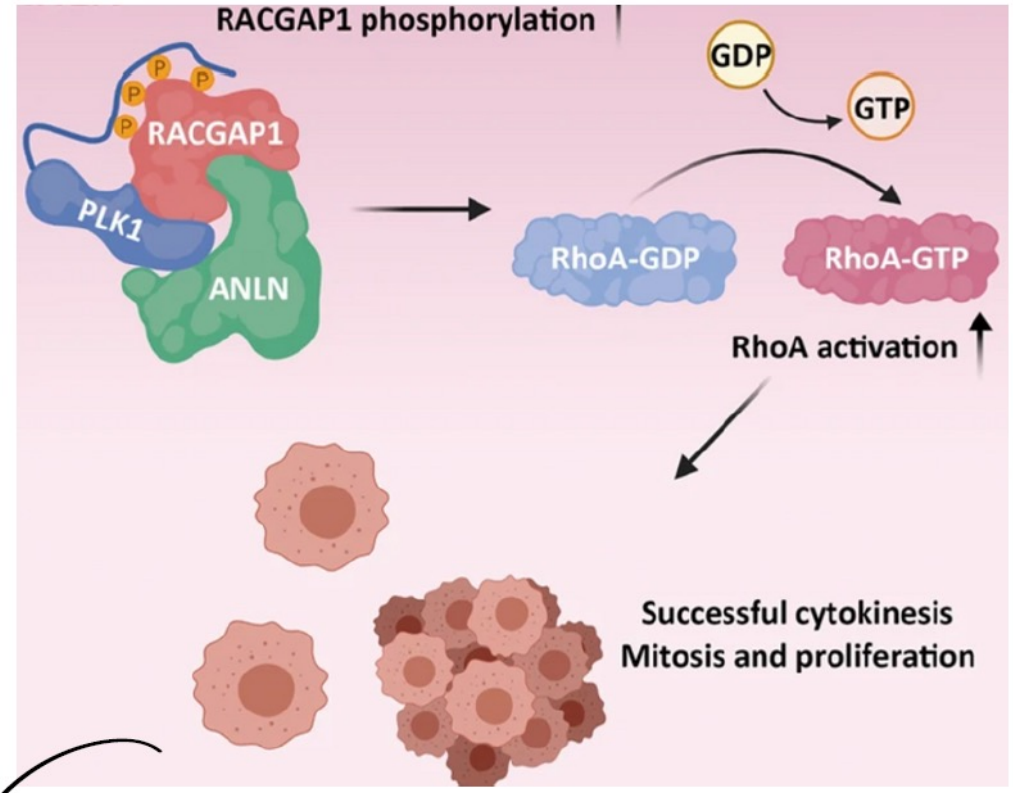
Hepatic cancer – RACGAP1

RACGAP1 competitive inhibition in hepatocellular carcinoma via vector based mRNA transfection

Lavinia Pace
Antonio Duarte
Ernest Serra
Miriana Santacroce
Luigi Fanelli



RACGAP1 3D structure
via Uniprot



RACGAP1 phosphorylation activation
cascade

https://www.nature.com/articles/s41388-022-02274-1#Sec13

Cancer

Group C: Belvedere, Jeong, Majaliwa, Virgilio

Retinoblastoma – rb1

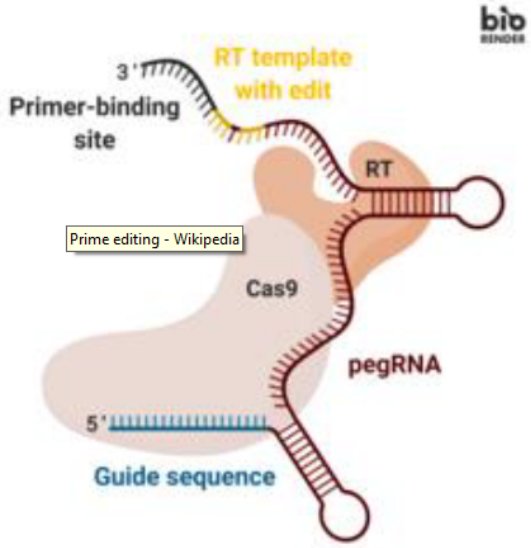
Prime editing of Rb1 gene as an innovative therapeutical strategy for retinoblastoma treatment

Background:

- Retinoblastoma is the most common type of eye cancer in children associated with the mutation in the RB1 gene.
- Every form of retinoblastoma (familial and sporadic) has the Rb1 gene mutated to some extent.
- Rb1 gene is located on chromosome 13, it consists of 27 exons.

Aim of the project: to develop a tool to prevent and cure the retinoblastoma onset

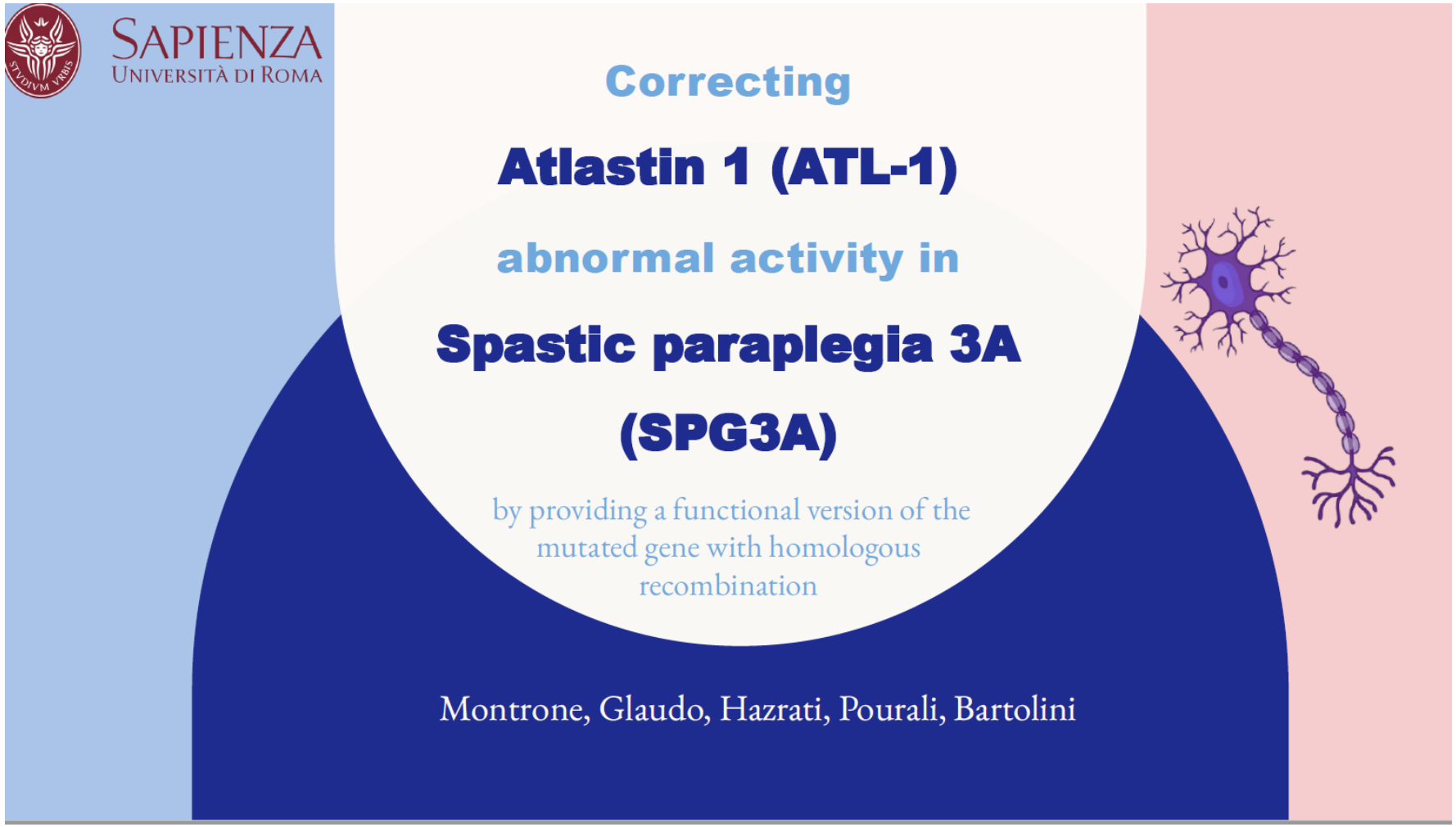
Strategy: design a pegRNA able to target a specific point mutation and stimulate the DNA repair systems.



Aging

Group B: Hazrati, Bartolini, Glaudo, Montrone, Pourali

Spastic paraplegias 3a – atl1



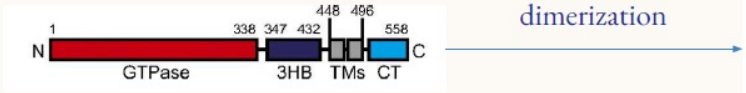
Aging

Group B: Hazrati, Bartolini, Glaudo, Montrone, Pourali

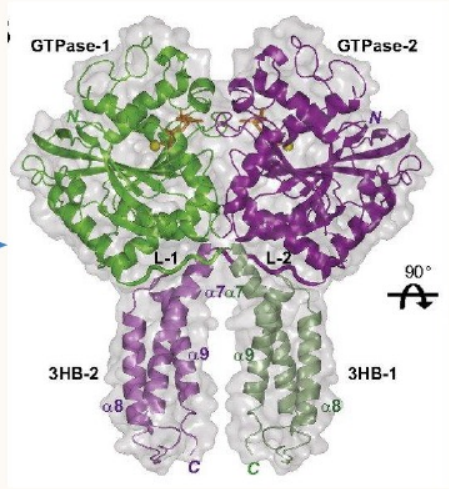
Spastic paraplegias 3a – atl1

Background - Spastic paraplegia 3A

- The **pure hereditary spastic paraplegias (HSPs)** are a group of conditions in which there is a progressive length-dependent degeneration of the distal ends of the corticospinal tract axons, resulting in spastic paralysis of the legs.
- **Autosomal dominant spastic paraplegia-3A (SPG3A)** is caused by heterozygous mutation in the ATL1 gene on chromosome 14q22. These lead to an abnormal activity of atlastin-1, which impairs the functioning of neurons.
- The ATL1 gene encodes **atlastin-1**, a dynamin-related GTPase, which plays a role in formation of the tubular endoplasmic reticulum (ER) network and in axon elongation in neurons.



dimerization



(Xin Bian et al., Structures of the atlastin GTPase provide insight into homotypic fusion of endoplasmic reticulum membranes, 2011)

Aging

Group A: Bernardi, Ilie, Colonnelli, Bastianelli

Charcot marie tooth – pmp22

Silencing of PMP22 promoter 2 using a CRISPR/Cas9 combined with methyltransferase (DNMT3A) in CMT1A

Aging

Group A: Bernardi, Ilie, Colonnelli, Bastianelli

Charcot marie tooth – pmp22

Does an epigenetic modification, at the level of PMP22 promoter 2, restore physiological phenotype of the SC?

- Overexpression of PMP22 protein overloads the protein folding apparatus in Schwann cells and activates the unfolded protein response. This leads to Schwann cell apoptosis, dys- and de- myelination and secondary axonal degeneration, ultimately causing neurological disabilities.

- CRISPR/dCas9-Dnmt3a-mediated targeted DNA methylation of PMP22 promoter 2.

- EXPECTED: restore of the physiological phenotype in SC and consequently the recovery of neuromuscolar function.

Aging

Group A: Bernardi, Ilie, Colonnelli, Bastianelli

Charcot marie tooth – pmp22

Silencing of P2X7 using a CRISPR/Cas9 combined with methyltransferase (DNMT3A) in CMT1A

Aging

Group A: Bernardi, Ilie, Colonnelli, Bastianelli

Charcot marie tooth – pmp22

Could inhibition of P2X7 gene restore the physiological phenotype of SC?

- High levels of intracellular [Ca2+] have been linked to the overexpression of the P2X7 purinoceptor/ion channel typical of CMT1A SCs. It has been reported that high intracellular Ca2+ led to the inhibition of SC maturation and differentiation, as well as inhibiting the expression and compaction of the myelin protein.
- CRISPR/dCas9-Dnmt3a-mediated targeted DNA methylation of P2X7
- EXPECTE: The inhibition of P2X7 causes a decrease in intracellular [Ca2+] and restore the physiological phenotype of SC