***DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO***

**XXXIX Cycle**

**Project proposal for a PhD scholarship (with no financial support from Sapienza)**

**Title of the research:**

Aberrant chromatin-nuclear lamina interactions in stromal cells as underlying mechanism of rhabdomyosarcoma initiation

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**Summary**

Spatial genome organization is crucial to maintain proper gene expression patterns in a cell-type specific manner. Silent heterochromatin is spatially confined towards the nuclear periphery and anchored to the nuclear lamina (NL) by lamina-associated domains (LADs), in which alternate fate genes are embedded to maintain cell identity. However, how heterochromatic lamina-associated regions are inherited after every cell division to maintain cell identity and whether re-establishment of peripheral heterochromatin organization is disrupted as a pre-requisite of malignant cell transformation is poorly studied. We have recently shown that the identity of skeletal muscle-resident PDGFRα+ mesenchymal progenitors (MPs) is controlled by PRDM16, a nuclear envelope protein that acts as an anchor for H3K9me2-marked chromatin at the NL to mediate LADs organization. Our results indicate that loss of PRDM16 impairs proper LADs establishment, inducing nuclear abnormalities and aneuploidy in MPs that derail their fate towards a myoblastic stage.

Since MPs are possible candidates as cells of origin, at least of a subset of rhabdomyosarcomas (RMS) and *PRDM16* has been recently reported among the recurrent deletions in RMS, we hypothesize that PRDM16 loss of function predisposes MPs to myoblastic transformation by impairing stable LADs-NL interaction.

To test our hypothesis and to understand how this is achieved, we aim:

- to study if PRDM16 mediates LADs-NL interactions, genome integrity and proper genome segregation during MPs’ cell cycle progression

- to investigate if PRDM16 ablation in MPs promotes RMS-genesis in vivo.

The results gained by our project have the potential to uncover a precise molecular and cellular context that allow RMS development, having the potential to identify a precise RMS subset. Moreover, our data showing PRDM16 as a regulator of heterochromatin assembly at the nuclear lamina, might inspire future research avenues for those working in other cancers in which PRDM16 is frequently mutated and inactivated.

**Pertinent Publications of the proponent (last 5 years)**

1. Dattilo D., Di Timoteo G., Setti A., Giuliani A., Peruzzi G., Beltran M, Centron-Broco A., Mariani D., **Mozzetta C**., Bozzoni I. The m6A reader YTHDC1 and the RNA helicase DDX5 promote the production of rhabdomyosarcoma-enriched circRNAs. ***Nature Communications*** *2023 Apr 5;14(1):1898. doi: 10.1038/s41467-023-37578-7.*
2. Gualtieri A., Bianconi V. Licursi V., Renzini A., Pieroni L., **Mozzetta C.** The RNA helicase DDX5 cooperates with EHMT2 to sustain alveolar rhabdomyosarcoma growth. ***Cell Reports,*** 2022,*40*, Aug 30th. Doi.org/10.1016/j.celrep.2022.111267
3. Randazzo P., Sinisi R., Gornati D., Bertuolo S., Bencheva L., De Matteo M., Nibbio M., Monteagudo E., Turcano L., Bianconi V., Peruzzi G., Summa V., Bresciani A., **Mozzetta C**., Di Fabio R. Identification and in vitro characterization of a new series of potent and highly selective G9a inhibitors as novel anti-fibroadipogenic agents. ***Bioorganic and Medicinal Chemistry Letters***, 2022 Sept 15. Doi: 10.1016/j.bmcl.2022.128858
4. De Stefano M.E., Ferretti V, **Mozzetta C**. Synaptic alterations as a neurodevelopmental trait of Duchenne muscular dystrophy. ***Neurobiology of Disease***. 2022 Jun 15;168:105718. doi: 10.1016/j.nbd.2022.105718.
5. Bianconi V. and **Mozzetta C.** Epigenetic control of muscle stem cells: time for a new dimension.***Trends in Genetics****, 2022 Jan 22:S0168-9525(22)00001-4. doi: 10.1016/j.tig.2022.01.001. Invited review.*
6. Macino M., Biferali B., Cipriano A., Ballarino M, **Mozzetta C.** Targeting the Expression of Long Noncoding RNAs in Murine Satellite Cells from Single Myofibers. ***Bio Protoc.*** 2021 Nov 5;11(21):e4209.
7. Biferali B., Bianconi V., Fernandez Perez D., Pöhle Kronawitter S., Marullo F., Maggio R., Santini T., Polverino F., Biagioni S., Summa V., Toniatti C., Pasini D., Stricker S., Di Fabio R., Chiacchiera F., Peruzzi G., **Mozzetta C.** Prdm16-mediated H3K9 methylation controls Fibro-Adipogenic Progenitors identity during skeletal muscle repair. ***Science Advances 2021, 7(23****), eabd9371*
8. Cipriano A., Macino M., Bonaiuto G., Santini T., Biferali B., Peruzzi G., Colantoni A., **Mozzetta C§**, Ballarino M**§**. Epigenetic regulation of Wnt7b expression by the cis-acting long noncoding RNA lnc-Rewind in muscle stem cells. ***Elife*** *2021****,*** *10, pp. 1–25, e54782*. **§** co-corresponding.
9. Pegoli G., Lucini F., **Mozzetta C§,** Lanzuolo C#. Single Myofiber Isolation and Culture from a Murine Model of Emery-Dreifuss Muscular Dystrophy in Early Post-Natal Development. ***Jove*** 2020; doi:10.3791/61516. §co-corresponding
10. Bianchi A.\*, **Mozzetta C.\*,** Pegoli G., Lucini F., Valsoni S., Rosti V., Petrini C., Cortesi A., Gregoretti F., Antonelli L., Oliva G., De Bardi M., Rizzi R., Bodega B., Pasini D., Ferrari F., Bearzi C., Lanzuolo C. Polycomb dysfunctional transcriptional repression contributes to Lamin A/C dependent muscular dystrophy. ***Journal of Clinical Investigation.*** 2020 doi:10.1172/JCI128161 \* equal.
11. Chiacchiera F, Morey L, **Mozzetta C.** Editorial: Epigenetic Regulation of Stem Cell Plasticity in Tissue Regeneration and Disease. ***Front Cell Dev Biol*** 2020 doi: 10.3389/fcell.2020.00082.
12. **Mozzetta C§**Tedesco FS. Challenging the "chromatin hypothesis" of cardiac laminopathies with LMNA mutant iPS cells.***J Cell Biol****.* 2019 doi: 10.1083/jcb.201907166. **§** corr. Author
13. Biferali B., Proietti D., **Mozzetta C. §** and Madaro L.**§**. Fibro-Adipogenic Progenitors (FAPs) cross-talk in skeletal muscle: the social network. ***Frontiers Physiology*** *2019; doi: 10.3389/fphys.2019.01074.* **§** co-corresponding

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4. Tremblay, A.M., et al., *The Hippo transducer YAP1 transforms activated satellite cells and is a potent effector of embryonal rhabdomyosarcoma formation.* Cancer Cell, 2014. **26**(2): p. 273-87.

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8. Drummond, C.J., et al., *Hedgehog Pathway Drives Fusion-Negative Rhabdomyosarcoma Initiated From Non-myogenic Endothelial Progenitors.* Cancer Cell, 2018. **33**(1): p. 108-124 e5.

9. Lisboa, S., et al., *Genetic diagnosis of alveolar rhabdomyosarcoma in the bone marrow of a patient without evidence of primary tumor.* Pediatr Blood Cancer, 2008. **51**(4): p. 554-7.

10. Shinkoda, Y., et al., *Rhabdomyosarcoma masquerading as acute leukemia.* Pediatr Blood Cancer, 2009. **52**(2): p. 286-7.

11. Ehnman, M., et al., *Distinct effects of ligand-induced PDGFRalpha and PDGFRbeta signaling in the human rhabdomyosarcoma tumor cell and stroma cell compartments.* Cancer Res, 2013. **73**(7): p. 2139-49.

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13. Joe, A.W., et al., *Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis.* Nat Cell Biol, 2010. **12**(2): p. 153-63.

14. Uezumi, A., et al., *Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle.* Nat Cell Biol, 2010. **12**(2): p. 143-52.

15. Biferali, B., et al., *Prdm16-mediated H3K9 methylation controls fibro-adipogenic progenitors identity during skeletal muscle repair.* Sci Adv, 2021. **7**(23).

16. Seki, M., et al., *Integrated genetic and epigenetic analysis defines novel molecular subgroups in rhabdomyosarcoma.* Nat Commun, 2015. **6**: p. 7557.

17. van Schaik, T., et al., *Cell cycle dynamics of lamina-associated DNA.* EMBO Rep, 2020. **21**(11): p. e50636.

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