Corso di dottorato in Scienze Biomolecolari
PhD in Biomolecular Sciences
Ciclo 39 / Cycle 39
A.Y. 2023-2024

Reserved scholarship G

Progetti finanziati nell'ambito dei Dipartimenti di Eccellenza 2023-2027.
Curriculum Biologia Quantitativa
Il vincitore sceglierà il progetto di ricerca dall'elenco sottostante.

MUR-funded grants - Departments of Excellence 2023-2027.
Quantitative Biology Curriculum
The winner will choose from the list available below the research project.

Principal Investigator	Project title
1 - Enrico Domenici & Nicola Segata	*Investigating the genetic basis of microbiome composition, function and transmission*
2 - Alessandro Romanel & Andrea Lunardi	*Mapping RNA splicing events at single cell level to identify Tumor Associated Antigens exposed by therapy resistant ERG+ prostate cells*
3 - Toma Tebaldi & Giovanni Piccoli	*Molecular and cell-specific mechanisms of sex unbalance in Parkinson's disease*

Project 1

Investigating the genetic basis of microbiome composition, function and transmission

Laboratory:
Laboratory of Neurogenomic Biomarkers (https://www.cibio.unitn.it/302/laboratory-of-neurogenomic-biomarkers)
Laboratory of Computational Metagenomics (https://www.cibio.unitn.it/147/laboratory-of-computational-metagenomics)

Principal Investigator: Enrico Domenici & Nicola Segata

Synthetic description of the activity and expected research outcome
The oral microbiome is a complex ecosystem which has been shown to play a role in health and disease, due to tight host-microbe interactions occurring at the oral mucosa having an influence on host metabolism and physiology.
Notably, recent genome wide associations study focused on the gut microbiome have shown that host genetic variations impact microbiome composition and function across human body sites. In turn, the microbiome composition can impact on health and disease either directly, e.g. through their metabolites, or indirectly, e.g. by altering host gene and protein expression. Based on the increasing availability of metagenomic data on large scale cohorts, genome-wide associations may therefore help to dissect the heterogeneity of the microbiome based on the genomic portrait of individual subjects, and provide information about health status and disease risk.
Within the context of a larger project aimed at fully characterizing the oral microbiome and its interplay with complex diseases in large cohorts, the PhD project will aim at performing large-scale association of the oral microbiome taxa and function with host genetic variants by integrating salivary metagenomic and human data from whole genome sequences. Given the availability of data on the transmission of the oral microbiome within families based on metagenomic strain-profiling, the genetic contribution to microbiome transmission will also be investigated. Finally, we will employ a novel pipeline for the extraction of viral genomes from metagenomic data to investigate for the first time the host genomic contribution to the viral microbiome.
Given the large sample size and the depth of the sequencing data, our work can set the basis for a characterization of the oral microbiome with unprecedented resolution. The resulting metagenome-GWAS will provide a framework for understanding the genetic regulation of the oral microbiome on a large scale, and sets the basis to investigate the causal role of the oral microbiome in disease development.

Candidate's profile (skills and competencies)
The ideal candidate will have:
• Master of Science degree in Bioinformatics, Computational Biology, Biotechnology (or equivalent)
• good programming skills
• knowledge in biostatistics or data science
• good communication skills (written and oral) and self-motivation
• very good knowledge of the English language.
• publications and experience in machine learning methods, and previous experience in the analysis of large genomic data are desirable.

Project 2

Mapping RNA splicing events at single cell level to identify Tumor Associated Antigens exposed by therapy resistant ERG+ prostate cells

Laboratory:
Laboratory of Bioinformatics and Computational Genomics (https://www.cibio.unitn.it/785/laboratory-of-bioinformatics-and-computational-genomics)
Armenise-Harvard Laboratory of Cancer Biology & Genetics (https://www.cibio.unitn.it/87/armenise-harvard-laboratory-of-cancer-biology-genetics)

Principal Investigator: Alessandro Romanel & Andrea Lunardi

Synthetic description of the activity and expected research outcome
Rationale: Because of its poor inflammatory profile and the very low tumor mutational burden, which are seals of excellent for tumor immunogenicity (1), Prostate Cancer is considered the prototype of a "cold tumor" and considered poorly suited for innovative clinical strategies aimed at stimulating antitumor immunity (2). However, both dogmas are slowly melting away, making room for interesting new therapeutic strategies for cold tumors. Firstly, common oncological treatments such as radio/chemotherapy have been demonstrated triggering a robust inflammatory condition inside the tumor mass (3-4). Secondly, aberrant RNA splicing in cancer cells leads to the generation of an unexpected amount of Tumor Associated Antigens with immunogenic potential (5). In line with this new perspective, we recently undertook a project aimed at warming up cold prostate tumors.

Preliminary results: Immortalized RWPE-1 prostate cells were genetically manipulated to express, under the control of doxycycline, the N-terminal deleted form of the transcription factor ERGMet40, the most frequent molecular alteration described in human prostate tumors (6). ERG+(doxy) and ERG-(mock) RWPE1 cells were treated with IR (10 Gy) and then frozen 96 hours after treatment. A similar amount of untreated ERG+(doxy) and ERG-(mock) RWPE1 were also collected. All the four conditions were sent to Dr Ternette at the Nuffield Department of Medicine of the University of Oxford (UK) for the MS analysis of the HLA-I epitome. An average of 4000 antigens have been univocally identified for each condition. Among them, classical TAAs (e.g., Testis Antigens) were isolated and classified based on the robustness of their identification in biological replicates and the statistical significance of their association with ERG expression in prostate cells. The hundreds of peptides that cannot be uniquely identified by MS analysis -the dark matter- represent a very precious source of TAA, since it is very likely that a considerable amount of them is generated by aberrant splicing events associated with ERG transcriptional activity.
Aims: Taking advantage of a newly generated MS data, this project aims at:
1. Mapping ERG-associated aberrant RNA splicing events at single cell level by leveraging the ICELL8 cx technology present at the Department CIBIO, which enables the generation of full- length scRNA-seq data. In particular, innovative computational methods for the identification and characterization of differential mRNA alternative splicing events that combine and integrate bulk RNA-seq and scRNA-seq data will be designed and developed;
2. Exploiting the RNA splicing data to investigate the HLA-I epitome of the ERG genetic model looking for ERG-associated splice-dependent TAA;
3. Identifying signatures of ERG-associated splice-dependent TAA exposed by PCa subclones showing de novo resistant to standard-of-care treatments (e.g., IR). In particular, computational methods for the integration of differential splicing and differential expression data will be explored to systematically identify resistant subclones carrying specific TTA patterns.
Future perspectives: Identification of canonical and noncanonical TAA signatures associated with ERG expression in prostate cells will help design targeted immunological strategies to improve eradication of therapy-resistant PCa clones by the immune system.
References:
(1) Duan Q, Zhang H, Zheng J, Zhang L. Turning Cold into Hot: Firing up the Tumor Microenvironment. Trends Cancer. 2020 Jul;6(7):605-618. doi:10.1016/j.trecan.2020.02.022. Epub 2020 Mar 21.PMID: 32610070. (2) Rebello RJ, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, Gillessen S, Van der Kwast T, Bristow RG. Prostate cancer. Nat Rev Dis Primers. 2021 Feb 4;7(1):9. doi: 10.1038/s41572-020-00243-0.PMID: 33542230. (3) Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51-72. doi: 10.1146/annurev-immunol-032712-100008. Epub 2012 Nov 12. PMID: 23157435. (4) Di Maggio FM, Minafra L, Forte GI, Cammarata FP, Lio D, Messa C, Gilardi MC, Bravatà V. Portrait of inflammatory response to ionizing radiation treatment. J Inflamm (Lond). 2015 Feb 18;12:14. doi: 10.1186/s12950-015-0058-3. eCollection 2015.PMID: 25705130. (5) Frankiw L, Baltimore D, Li G. Alternative mRNA splicing in cancer immunotherapy. Nat Rev Immunol. 2019 Nov;19(11):675-687. doi: 10.1038/s41577-019-0195-7. Epub 2019 Jul 30. PMID: 31363190. (6) Alaimo A, Lorenzoni M, Ambrosino P, Bertossi A, Bisio A, Macchia A, Zoni E, Genovesi S, Cambuli F, Foletto V, De Felice D, Soldovieri MV, Mosca I, Gandolfi F, Brunelli M, Petris G, Cereseto A, Villarroel A, Thalmann G, Carbone FG, Kruithof-de Julio M, Barbareschi M, Romanel A, Taglialatela M, Lunardi A. Calcium cytotoxicity sensitizes prostate cancer cells to standard-of-care treatments for locally advanced tumors. Cell Death Dis. 2020 Dec 7;11(12):1039. doi: 10.1038/s41419-020-03256-5. PMID: 33288740

Candidate's profile (skills and competencies)
The ideal candidate studied bioinformatics or computational biology, has analytical and quantitative analysis skills, excellent programming skills (C, Python, Bash scripting), is knowledgeable of human genomics and cancer genomics and has experience in management and analysis of next-generation sequencing data.

Project 3

Molecular and cell-specific mechanisms of sex unbalance in Parkinson's disease

Laboratory:
Laboratory of RNA and Disease Data Science (https://www.cibio.unitn.it/1349/laboratory-of-rna-and-disease-data-science)
Dulbecco Telethon Laboratory of Biology of Synapses (https://www.cibio.unitn.it/304/dulbecco-telethon-laboratory-of-biology-of-synapses)

Principal Investigator: Toma Tebaldi & Giovanni Piccoli

Synthetic description of the activity and expected research outcome
Epidemiological and biological data suggest the existence of a sexual dimorphism in Parkinson's disease (PD) where women show a lower risk of disease development and a tendency to later disease onset [1]. Estrogen neuroprotective effects have been widely reported in a number of neuronal cell systems, including the nigrostriatal dopaminergic neurons [2]. ER positively modulates the autophagy lysosomal pathway (ALP) [7,8].. Several genes mutated in familial forms of PD are linked to ALP, including LRRK2 [5,6]. LRRK2 is a multidomain protein with kinase and GTPase activities [7], mainly functioning in the endosome recycling system, including autophagy and lysosome biology [8]. Recently, we reported that mice overexpressing human LRRK2 G2019S (hG2019S) exhibit an age dependent motor dysfunction and

neuronal apoptosis in the substantia nigra pars compacta caused by the toxic accumulation of protein aggregates. We have characterized the sex impact in our hG2019S mouse line. We found that only male hG2019S animals show overt motor impairment and protein aggregation at 6 months. Noteworthy, we observed a significant difference in terms of basal autophagic activity in primary neurons prepared from P1 male or female transgenic mice.

Objectives

We aim to gain insights into the gender differences underpinning ALP dysfunction in PD, building on the sex effect observed in our hG2019S LRRK2 mice. We will dissect ALP defects in males vs. females using single cell RNA-seq approaches (Aim 1) and we will validate prominent hits in complementary disease relevant models (Aim 2). Aim 1 (months 1-18). We will examine the transcriptional profile differences in wild type and hG2019S males and females mice by scRNA-seq. We will sacrifice the animals at 6 months according to national guidelines. At least 6 animals for experimental group will be used as tissue donors. We will collect the region of 1.28 mm bregma to 2.28 mm bregma containing the SNpc by using a brain matrix (1 mm slices). For each genotype and sex, we will pool and process about 5 millions of cells. We will generate sequencing libraries using 10X Genomics Chromium technology with 3' end gene expression library preparation. The analysis will be performed first on an Illumina NextSeq 500 SBS sequencing machine as in 9. In parallel we will also consult existing single cell and spatial transcriptomic datasets to gain preliminary and integrative information on relevant marker genes and modules . We will isolate relevant midbrain areas. Single-cell resolution will be determinant to identify sex unbalance mechanisms in specific subpopulations of cells and to identify cell-specific critical genes. Eventually, we will exploit alternative single-cell platforms such as Takara ICELL8 to gain improved coverage and sequencing depth on a selection of cells present in specific areas identified from the previous analysis. Data from each sample will be analysed using conventional pipelines the Seurat R package as in [10] and we will compare our results with published single-cell atlases, such as the recent atlas of the human SN [11]. Milestone 1, expected month 18: scRNA-seq atlas of male vs. female, WT vs. hG2019S mouse midbrains

Aim 2 (months 19-36). We will select genes that are differentially expressed across sex and genotype. First, we will focus on genes involved in ALP. Our validation work-flow will start from mice brain specimen where we will validate mRNA levels of the top 10 hits by digital PCR. Positive and relevant candidates will be further characterized at the protein level in the PSCs from male and female LRRK2 G2019S patients and the corresponding gene corrected lines are already available in our lab [12] and have been already fully characterized [13]. The generation of DANs and astrocytes will be performed as described [14,15] and assessed by q RT-PCR and IF for specific markers as in [12].

Milestone 2, expected month 36: ALP relevant cell- and sex-specific genes validated

References

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6. Blauwendraat, C. et al. Frequency of Loss of Function Variants in LRRK2 in Parkinson Disease. JAMA Neurol 75, 1416–1422 (2018).
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10. Hao, Y. et al. Integrated analysis of multimodal single-cell data. Cell 184, 3573-3587.e29 (2021).
11. Agarwal, D. et al. A single-cell atlas of the human substantia nigra reveals cell-specific pathways associated with neurological disorders. Nat Commun 11, 4183 (2020).
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13. Reinhardt, P. et al. Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. Cell Stem Cell 12, 354–367 (2013).
14. Booth, H. D. E. et al. RNA sequencing reveals MMP2 and TGFB1 downregulation in LRRK2 G2019S Parkinson's iPSC-derived astrocytes. Neurobiology of Disease 129, 56–66 (2019).
15. Reinhardt, P. et al. Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. PLoS ONE 8, e59252 (2013).

Candidate's profile (skills and competencies)

The ideal candidate has either 1) previous experience in computational biology and she/he is eager to acquire wet-bench related expertise, or 2) he/she has competence in molecular and cellular biology and wants to learn computational biology approaches for analysis of single cell data.