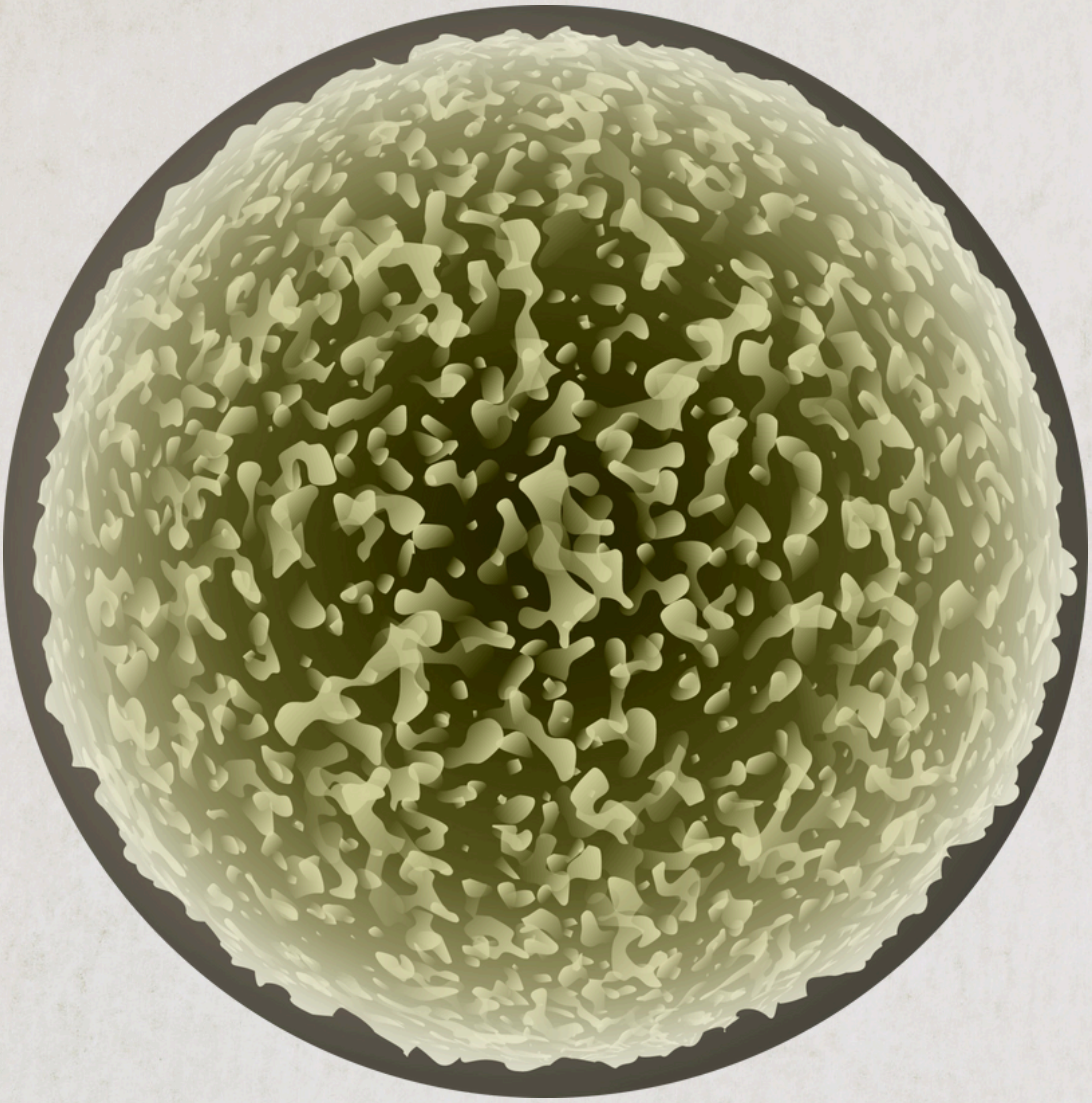
Single Cell LATAM Symposium



CONNECTING COMMUNITIES & ADVANCING RESEARCH

RIO DE JANEIRO
16 - 17 AUGUST, 2024

INCA
INSTITUTO NACIONAL DE CÂNCER

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Single Cell LATAM Symposium: Connecting Communities and Advancing Research

Brazilian National Institute of Cancer, Rio de Janeiro, RJ, Brazil
16-17 August 2024

Organisers

David Adams
Wellcome Sanger Institute, UK

Mariana Boroni
Instituto Nacional de Câncer, Brazil

Patricia Possik
Instituto Nacional de Câncer, Brazil

Scientific Programme Committee

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Danielle Bonfim
Universidade Federal do Rio de Janeiro, Brazil

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Universidad de Costa Rica, Costa Rica

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Dear Attendee,

This event was organised by the Brazilian National Cancer Institute (INCA) in collaboration with Wellcome Connecting Science (WCS). INCA is committed to consolidating the Oncological Care Network, which aims to generate, disseminate, articulate and implement cancer care policies and actions. WCS funds, develops and delivers training and conferences that span basic research, cutting-edge biomedicine and the application of genomics to healthcare.

Over the past decade, single-cell genomics has witnessed rapid advancement, fundamentally altering how we approach and resolve biological inquiries. The ability to examine individual cells has unveiled gene expression dynamics and cellular diversity previously obscured in aggregate analysis. However, with the simultaneous profiling of millions of cells, challenges arise in experimental design, mitigating errors and biases, and handling extensive datasets. Nevertheless, this scenario is more demanding in Latin America (LATAM) countries, which face challenges due to limited infrastructure, funding constraints, and a shortage of expertise hindering the adoption of these advanced technologies.

To overcome the challenges in LATAM and promote a collaborative approach between countries, the Single Cell LATAM Symposium aims to bring together local researchers interested in single-cell technologies to discuss the latest key approaches in single-cell genomics while exploring questions that are distinctive to LATAM, such as challenges, opportunities, and how to improve capacity building in Single-Cell Genomics within the LATAM community. Spanning two days, and 18 hours of contact, the symposium is organised to cover a wide array of topics crucial for both newcomers and seasoned researchers in the field, including talks on the latest technological advances, dataset integration strategies, and the impacts of single-cell technologies on various diseases. The symposium also includes poster sessions and fosters informal interactions among LATAM experts, who are working at the forefront of the single-cell field, with researchers at all career stages.

Best wishes,

Single Cell Symposium Organisers and Scientific Committee

Acknowledgments

We would like to acknowledge the support of the following organisations for the Single Cell LATAM Symposium: Connecting Communities and Advancing Research 2024.

SCIENTIFIC PROGRAM

Single Cell LATAM Symposium: Connecting Communities and Advancing Research - 16-17 August 2024
Hilton Copacabana Hotel, Rio de Janeiro, Brazil

Friday 16 august 2024			Day 1: Establishing the Landscape and Setting Expectations
Start (GMT -3)	**Finish (GMT -3)**	**Time allocated**	**Presenter details**
11:30	12:00	30 min	Transfer to Symposium Venue
12:00	**13:00**	**1 hour**	**Registration and Lunch**
13:00	**13:15**	**15 min**	**Opening Remarks**
		5 min	**Welcome** *Speaker: Liã Bárbara Arruda, Wellcome Connecting Science, United Kingdom*
		10 min	**Establishing the Landscape and Setting Expectations** *Speaker: Patricia Possik, INCA, Brazil*
13:15	**13:55**	**40 min**	**Keynote Talk 1**
			Chair: Mariana Boroni, INCA, Brazil
		30 min	**Single-Cell RNA Sequencing Reveals Adipose Tissue Macrophage Subpopulations Associated with Metabolic Disease in Humans** *Marcelo Alves da Silva Mori, University of Campinas, Brazil*
		10 min Q&A	
13:55	**14:55**	**60 min**	**Session 1: Diseases in the Era of Single-Cell Methodologies**
			Chair: Alvaro Lladser, Fundacion Ciencia y Vida, Chile
		15 min	**Regulation of Th17 cells by TMEM176B: implications for immunotherapy and disease** *Natalia Rego, Institut Pasteur de Montevideo, Uruguay*
		15 min	**Functional Screen of Identity Determinants in the Developing Mouse Cerebral Cortex** *Daniela Di Bella, Harvard University, USA*
		15 min	**Understanding Functional Reprogramming in Human Metastasis Through Single-Cell Genomics** *Hugo Gonzales, Fundacion Ciencia Para La Vida, Chile*
		15 min Q&A	
14:55	**15:25**	**30 min**	**Sponsor Session 1: Pensabio**
			Chair: Liã Bárbara Arruda, Wellcome Connecting Science, United Kingdom
		20 min	**Unlock the mysteries of biology with Single-Cell Multi-Omics and Spatial Transcriptome** *Natalia Fernandes Garcia de Carvalho, Pensabio, Brazil*
		10 min Q&A	
15:25	15:55	30 min	**Coffee Break**
15:55	**16:25**	**30 min**	**Chan Zuckerberg Initiative**
			Chair: Danielle Bonfim, Universidade Federal do Rio de Janeiro
		25 min	**Exploring the Frontiers of Single-Cell Genomics with CELLxGENE: Interactive Visualization & Analysis in Action** *Maximilian Lombardo, Chan Zuckerberg Initiative, USA*
		5 min Q&A	
16:25	**17:05**	**40 min**	**Lightning Talks 1**
			Chair: Isabela Malta, Wellcome Connecting Science, United Kingdom
		5 min	**Lightning talk 1: Unraveling the plasticity of differentiated mesenchymal stromal cells** *Alejandro Villa, Instituto Venezolano de Investigaciones Científicas, Venezuela*
		5 min	**Lightning talk 2: Understanding Neuroblastoma through the integration of single cell data** *Federico Garde, IQUIBICEN-CONICET, Argentina*
		5 min	**Lightning talk 3: Longitudinal Immune Analysis Differentiates Responder and Non-Responder Profiles in NSCLC Patients Treated with Pembrolizumab and Chemotherapy** *Thais Romano Ferreira, Center for Research in Immuno-oncology (CRIO) - Hospital Israelita Albert Einstein, Brazil*
		5 min	**Lightning talk 4: Differences in transcriptional signatures of peripheral immune mononuclear cells during Hantavirus Andesense infection** *Juan Calderon, Centro de Genética y Genómica - Universidad del Desarrollo, Chile*
		20 min Q&A	
17:05	**18:05**	**60 min**	**Roundtable 1: Improving Data Sharing and Governance Frameworks in LATAM Countries**
			Chair: Yesid Cuesta Astroz, University of Antioquía, Colombia
		15 min	**Talk: Data-Sharing and Good Governance: Anticipate to Innovate** *Ma'n Zawatti, Global Alliance for Genomics and Health (GA4GH) and McGill University, Canada*
		15 min	**Talk: Human Cell Atlas Data Structure and Governance** *Vinicius Maracaja-Coutinho, HCA Latam and University of Chile, Chile*

		30 min	*Discussion*
18:05	**18:10**	**5 min**	**Closing Remarks Day 1** *Speaker: Patricia Possik, INCA, Brazil*
18:10	**20:10**	**2 hours**	**Poster Session 1 and Networking**

Saturday 17 august 2024 — Day 2: Deep Diving into Applications and Capacity Building

Start (GMT -3)	Finish (GMT -3)	Time allocated	Presenter details
08:00	08:30	30 min	Transfer to Symposium Venue
09:00	**09:40**	**40 min**	**Keynote Talk 2** *Chair: Lucía Spangenberg, Institut Pasteur de Montevideo, Uruguay*
		30 min	**Deciphering the T cell networks underlying long-lasting immunity against solid tumors** *Alvaro Lladser, Fundacion Ciencia y Vida, Chile*
		10 min Q&A	
09:40	**10:20**	**40 min**	**Lightning Talks 2** *Chair: Isabela Malta, Wellcome Connecting Science*
		5 min	**Lightning talk 5: Exhausted T cells and M2 macrophages form progression-related spatial arrangements in tumors of patients with cancer-prone syndrome Fanconi Anemia** *Pablo Siliceo, Intituto de Investigacioens Biomédicas (IIBO), Universidad Nacional Autónoma de México (UNAM), Mexico*
		5 min	**Lightning talk 6: Cellular immunological phenotype in Guillain-Barré Syndrome (GBS): Anti-infective and autoreactive profile** *Melissa Solarte Cadavid, Biologist-PhD student/Universidad del Valle, Colombia*
		5 min	**Lightning talk 7: Single-cell Atlas Reveals Immunophenotypes Associated with Clinical Outcome in Pancreatic Cancer** *Gabriel Pozo, UFCSPA, Brazil*
		5 min	**Lightning talk 8: Defining single cell EMT signatures influencing patient outcome in colorectal cancer** *Ricardo Chinchilla-Monge, Centro de Investigación en Cirugía y Cáncer, Universidad de Costa Rica, Costa Rica*
		20 min Q&A	
10:20	**10:35**	**15min**	**Sponsor Session 2: New England Biolabs** *Chair: Liã Bárbara Arruda, Wellcome Connecting Science, United Kingdom*
		12 min	**Innovating NGS Workflows with NEBNext** *Alexander Rochette, New England Biolobs, Brazil*
		3 min Q&A	
10:35	11:05	30 min	Coffee break
11:05	**13:05**	**2 h**	**Workshop: Challenges for Single-Cell Sequencing in Latin America** *Chairs: David Adams, Wellcome Sanger Institute, United Kingdom and Ricardo Chinchilla-Monge, Centro de Investigación en Cirugía y Cáncer, Universidad de Costa Rica, Costa Rica* *Facilitator 1: Liã Bárbara Arruda, Wellcome Connecting Science, United Kingdom* *Facilitator 2: Lucía Spangenberg, Institut Pasteur de Montevideo, Uruguay* *Facilitator 3: Alvaro Lladser, Universidad San Sebastian, Chile* *Facilitator 4: Edith Kordon, Universidad de Buenos Aires, Argentina* *Facilitator 5: Danielle Bonfim, Universidade Federal do Rio de Janeiro, Brazil* *Facilitator 6: Patricia Possik, Instituto Nacional do Cancer (INCA), Brazil*
13:05	**15:05**	**2 hours**	**Poster Session 2 and Lunch**
15:05	**15:35**	**30 min**	**Sponsor Talk 3: Interprise** *Chair: Liã Bárbara Arruda, Wellcome Connecting Science, United Kingdom*
		20 min	**Solutions for single cell sorting: Scaling up from research up to bioproduction** *Luis Perin, Interprise, Brazil*
		10 min Q&A	
15:35	**15:55**	**20 min**	**Keynote Talk 3:** *Chair: Ricardo Chinchilla, Centro de Investigación en Cirugía y Cáncer (CICICA), Costa Rica*
		15 min	**The underrepresentation of Latin American samples in genomic studies: what is the problem, why does it exist and how can we solve it?** *Daniela Robles Espinoza, International Laboratory for Human Genome Research - UNAM, Mexico*
		5 min Q&A with speakers	
15:55	**16:25**	**30 min**	**Coffee break**
16:25	**17:25**	**60 min**	**Roundtable 2: Setting a Collaborative Agenda for the Next Decade** *Chair: Edith Kordon, Universidad de Buenos Aires, Argentina*
		15 min	Chan Zuckerberg Initiative: Empowering and Investing in Scientists in Latin America *Priscila Martinez, Chan Zuckerberg Initiative, USA*

		15 min	Integrating Latin America into the Global Human Cell Atlas Initiative *Patricia Severino, HCA LATAM and Albert Einstein Research and Education Institute, Brazil*
		30 min	*Discussion*
17:25	**17:40**	**15 min**	**Event closing** *Speaker: Mariana Boroni, INCA, Brazil*
17:40	**18:40**	**60 min**	**Light refreshments networking**

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Single Cell LATAM Symposium: Connecting Communities and Advancing Research 2024

Abstracts selected for oral presentations

Unraveling The Plasticity Of Differentiated Mesenchymal Stromal Cells

Villa Sifontes, Alejandro David[1]

[1]Unidad de Terapia Celular - Laboratorio de Patología Celular y Molecular, Centro de Medicina Regenerativa, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827, Caracas 1020-A, Venezuela.

It has been documented that when differentiated in other lineages (such as osteogenic and adipogenic), mesenchymal stromal cells (MSCs) appear to retain their immunoregulatory ability. That may be in part due to the heterogeneity in MSCs cultures, in which not all the cells may be able to differentiate, therefore maintain their immunomodulatory properties. Nonetheless, it may be possible that these differentiated cells may express a mixed phenotype that allows them to retain some degree of MSCs function. This notion is supported by the fact that MSCs have been found to exhibit differential expression profiles depending on their tissue location, even within the same donor. In relation to the above, the use of single cell sequencing (SCS) technology could aid in the identification of subpopulations of MSCs not only prone to differentiate into some particular cellular subtype, but also prone to exert a greater immunoregulatory effect and lower probability of rejection. By isolating human bone marrow-derived MSCs, the most frequently used source of MSCs for clinical trials, followed by the isolation of single cells, subsequent DNA/RNA extraction from individual cells can be performed in order to amplify the genetic material and construct a DNA and/or RNA library for subsequent high-throughput sequencing, after which the data can be analyzed. Just as there are functionally distinct subpopulations of undifferentiated MSCs, since SCS analysis has revealed that even cells from the same tissue source and donor comprise different clusters or cells subpopulations with different biological functions (such as stemness, functionality and proliferation), it is to be expected that after SCS analysis, functionally distinct populations of differentiated MSCs with different immunoregulatory, functional and proliferative capacity can be identified. If so, further analysis of the secretome of these subpopulations as well as their epigenetic profile could help to elucidate the factors that regulate this cellular heterogeneity.

Understanding Neuroblastoma Through The Integration Of Single Cell Data

Federico J. Garde1, Candela D. Pastore1, Daniela J. Di Bella2, Abel L. Carcagno1

1 Cell Differentiation and Cancer Laboratory, ETI1, IQUIBICEN-CONICET, FCEyN-UBA, CABA, Argentina. 2 Department of Stem Cell and Regenerative Biology, Harvard University, Boston, USA.

Neuroblastoma (NB) is the most common extracranial childhood tumor arising from the adrenal medulla. NB is a tumor derived from neural crest cells and is often considered a disease associated with defective cell differentiation. The generation of reference maps of primary NB tumors and the analysis of undifferentiated neural crest-derived cells present in the embryonic adrenal medulla by scRNAseq are invaluable tools for deciphering the heterogeneity and origin of these tumors. Our aims were: 1) Generate and analyze a unique reference map of neuroblastoma tumors by integrating publicly available single-cell RNA sequencing data. 2) Use this dataset to evaluate ASCL1 as a potential target for NB therapy as a complement of our in vitro results showing that ASCL1 silencing in NB induces cellular differentiation and results in impaired tumor aggressiveness We integrated data from Kildisiute et al, Dong et al, and Jansky et al. using Seurat-CCA integration and the resulting dataset was filtered to obtain the tumoral cells previously identified by inferCNV. Dimensionality reduction was performed on the obtained dataset and clusters were calculated by Uniform manifold approximation and projection (UMAP). Examining the expression of gene signatures specific to human fetal adrenal medulla cell types and aligning our dataset with scRNAseq data from the same organ using Seurat TransferID, we found that NB cells share a common origin, adrenal medulla` sympathoblasts and that heterogeneity can be explained by their grade of cellular differentiation and associated cell cycle. This was supported by the differentially enriched processes and pathways characterized by Gene Ontology and Reactome analyses and by trajectory analysis performed by combining CytoTrace potency score with Monocle3. Finally, we found that ASCL1 was mainly expressed in less differentiated cells, and non-negative matrix factorization revealed its co-expression with genes linked to early neurogenesis, cell migration, and proliferation.

Single-Cell Atlas Reveals Immunophenotypes Associated With Clinical Outcome In Pancreatic Cancer

Gabriel F. Pozo de Mattos Pereira1, Marvin Paulo Lins1, Alessandro Bersch Osvaldt2, Simone Marcia dos Santos Machado2, Cristina Cazabuena Bonorino1, Eduardo Cremomese Filippi-Chiela2,3

1 Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). 2 Hospital de Clínicas de Porto Alegre (HCPA). 3 Universidade Federal do Rio Grande do Sul (UFRGS)

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and lethal tumor. PDAC also shows poor response to many conventional treatments and is refractory to immunotherapy. We hypothesize that these challenges arise from the highly heterogeneous and immunosuppressive tumor microenvironment (TME) in PDAC. Therefore, to comprehend the players behind this immunosuppressive TME, we integrated 10 publicly available datasets into a robust PDAC scRNA atlas covering 201 patients (159 PDAC, 24 non-cancer, and 18 metastatic PDAC). The TME showed distinct composition for each tissue, predominating a fibro-inflammatory phenotype with infiltrating Tregs and dysfunctional CD8 T cells in primary PDACs. In contrast, adjacent normal tissue exhibited enrichment of memory CD8 T cells (FDR < 0.1). Metastatic PDAC was characterized by a reduction in stromal cells combined with myeloid and naive T cells infiltration. Comparison between treated and untreated PDAC revealed the enrichment of cancer-associated fibroblasts (CAFs) in treated patients. Besides, T cells from treated patients exhibited higher levels of heat shock genes which has been linked to immunotherapy resistance. Next, we stratified patients based on TME cell composition. Unsupervised clustering revealed 6 clusters: Desert (high tumor cell fraction); Fibrotic (high fraction of stromal cells); Normal-like (high fraction of acinar and normal ductal cells); M-enriched (high fraction of myeloid cells), Immune-CD8_high and Immune-CD8_low. The Immune-CD8_high cluster was associated with a good prognosis in a dataset (n=43) present in our atlas. Then, we performed bulk RNA deconvolution of the TCGA cohort (n=145) and recovered almost all (except for M-enriched) previously identified clusters. Survival analysis showed better overall survival for the Immune-CD8_high cluster ($p < 0.05$). Collectively, our study identifies distinct features of immune escape in PDAC associated with response to therapy and patient survival, reinforcing the need for better patient stratification.

Differences In Transcriptional Signatures Of Peripheral Immune Mononuclear Cells During Hantavirus Andesense Infection

Julio Santelices1,2; Juan Calderón3, Cecilia Vial2

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Hantavirus Cardiopulmonary syndrome (HCPS) is a zoonotic disease with one of the highest case fatality rates (30%). It is caused by a family of Hantaviruses endemic to the American continent. Particularly, the Andesense Species (ANDV) is present in Chile and Argentina and is the only known Hantavirus with person-to-person transmission, which confers pandemic potential. In more than 85% of cases the disease can rapidly progress towards HCPS without a clear and reliable indicator of clinical outcome. HCPS is characterized by a sudden failure of heart and lungs that can also affect hepatic and renal function. Treatment is based on critical care support, including but not limited to vasoactive drugs, mechanical ventilation and ECMO when cardiac function drops below a minimal threshold as reported in literature. However, a significant portion of patients present only with unspecific flu-like symptoms, including fever, headache, fatigue, myalgia, and gastrointestinal issues. A hallmark of disease severity is the loss of endothelial integrity and consequent capillary leakage. The underlying mechanisms are not completely elucidated but it is well-known that an exacerbated immune response that includes a cytokine storm are present in severe HCPS patients. We propose to characterize PBMCs of mild and severe HCPS patients by using single-cell RNA-seq. We hypothesize that there are significant differences in transcriptional signatures of immune mononuclear cells that are associated with each clinical course. We will select patients affected by ANDV that were recruited at early onset with mild symptoms and evolved to severe HCPS. PBMCs from patients that do not progress toward severe HCPS will be used as baseline. With these analyses we expect to describe the main cellular signatures discrepancies between mild and severe HCPS patients to elucidate part of the immune physiopathology
of HCPS.

Cellular Immunological Phenotype In Guillain-Barré Syndrome (Gbs): Anti-Infective And Autoreactive Profile

Nelson Rivera Franco1,2, Melissa Solarte Cadavid1,2, Diana López-Alvarez1,2,3, Erica M Aristizábal1,2 & Beatriz Parra1,2

1 Grupo de Investigación Virus Emergentes y Reemergentes, Universidad del Valle, Cali, Colombia. 2 Red Neurovirus Emergentes en Las Américas, Universidad del Valle, Cali, Colombia. 3 Departamento de Ciencias Biológicas, Universidad Nacional de Colombia, Palmira, Colombia.

Guillain-Barré syndrome (GBS) is an acute-onset immune-mediated polyradiculoneuropathy characterized by an aberrant autoimmune response directed to the peripheral nerves and their spinal roots, causing neuromuscular paralysis, due to an immune stimulation that is usually a previous infection, being Campylobacter jejuni responsible for at least one-third of these infections associated with GBS. Other associated infections include Mycoplasma pneumoniae and ZIKV. On the other hand, the immunologic mechanisms underlying this disease are not entirely clear. It appears that leukocyte infiltration of immunoprivileged tissue such as the peripheral nervous system plays a crucial role in the pathophysiology of this disease. This study aimed to elucidate the immune mechanisms associated with the pathogenesis of this post-infectious neurological complication. We focus on patients who have experienced infections such as ZIKV, Campylobacter jejuni, or Mycoplasma pneumoniae previous to the development of GBS. In this research, immunophenotyping of PBMC from these patients and controls will be performed by flow cytometry and single-cell RNA sequencing. An anti-infective and/or autoimmune phenotype are expected to be found associated with GBS cases. As previous results, four transcriptomes were analyzed with the RNA-seq immune analysis pipeline or RIMA, which is useful for molecular profiling and immunological characterization. The results obtained showed a higher frequency of memory TCD4+ cells (9%) and monocytes (57%) compared to other cell types or normal profiles. Although with the limitation of a very small sample size, these preliminary results agree with previous findings proposing the infiltration of T lymphocytes and macrophages that attack peripheral nerve cells generating demyelination. The marked increase of peripheral blood monocytes, which are the precursors of tissue macrophages, could be evaluated as a possible biomarker of tissue infiltration by macrophages in GBS and it is hoped that these preliminary results can be extended and validated by the application of flow cytometry and single-cell RNA-seq.

Exhausted T Cells And M2 Macrophages Form Progression-Related Spatial Arrangements In Tumors Of Patients With Cancer-Prone Syndrome Fanconi Anemia.

Pablo Siliceo-Portugal1,2, Ada Junquera-Mencia3, Luis A. Flores-Mejía1,2, Mahiru Trejo-León1, Fernando Pérez-Villatoro3, Anna Laury3, Renee Fisher4, Michael Epperly4, Aliesha González-Arenas1, Sara Frías1,5, Leonard Harris6,7,8, Joel Greenberger4, Eunike Velleuer9, Anniina Färkkilä3,10,11, Alfredo Rodríguez1,2

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Introduction: Fanconi anemia (FA) is a chromosome instability syndrome characterized by an increased predisposition to develop aggressive squamous cell carcinomas (SCC). Due to its underlying DNA repair deficiency, FA has been used as de facto model to study carcinogenesis and identify early mechanisms of tumor initiation and development.
Materials and methods: We performed multiplexed single-cell spatial analysis using whole-slide tissue cyclic immunofluorescence (t-CycIF) of 33 tissue slides cut from formalin-fixed paraffin-embedded (FFPE) anogenital and head and neck SCC tumor samples from both non-FA and FA patients, as well as wild type and FA mice treated orally with the potent carcinogen 4NQO. Slides underwent multiple cycles of immunofluorescent staining (t-CyCIF) and scanning (Rarecyte Finder II) with a panel of >20 fluorochrome-tagged antibodies.). The resulting cyclic scans were aligned and registered (ASHLAR) into a multidimensional image that was analyzed using state-of-the-art computational methods (SCIMAP, Napari, StarDist MCMICRO) in the context of their tumor region, carcinogenic progression, tumor-immune architecture, and functional interactions.
Result: We identified phenotypical heterogeneity among the annotated tumor regions, showing increased diversity of cellular neighborhoods that correlated with carcinogenic progression. In FA patients, there was an increase in cellular neighborhoods with DNA damage and an enrichment of tumor-associated macrophages and exhausted T cells along carcinogenic progression. In the 4NQO carcinogenesis mouse model, changes in the FA mouse epithelium appeared early (8 weeks), in comparison to wild type mice, including extensive fields of cells with DNA damage (H2AX) that expressed proliferation markers (Ki67), and infiltration of immune cells, which are in close contact with DNA damaged epithelial cells
Conclusion: We profiled the TME of FA SCCs through image-based single-cell proteomics and identified unique cellular neighborhoods along carcinogenic progression, where hypofunctional immune infiltration correlated with DNA damage presence in tumor cells and colocalization with immunosuppressive cell
types.

Defining single cell EMT signatures influencing patient outcome in colorectal cancer.

Alexis G. Murillo Carrasco1,2, Annie Cristhine M. S. Squiavinato3, Cristóvão de Lanna4, Daniela Bizinelli5, Danielle Carvalho3, David J. Adams6, Flavia Aguiar3, Patricia Possik3, Ricardo Chinchilla-Monge7 ,Silvana Pereyra8

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Abstract
Colorectal cancer (CRC) ranks as the third most frequently diagnosed malignancy and the second highest cause of cancer-related death (both sexes in 2022, Global Cancer Observatory). Although local and distant metastases are closely related to the poor prognosis of the disease, the specific mechanisms that drive this process are not fully understood. Tobetter understand epithelial-mesenchymal transition (EMT), we implemented a computational single-cell approach that integrates known EMT-related genes to identify clusters of cells participating in EMT. We then used these clusters to identify other relevant genes for EMT and prognosis. First, we gathered single-cell transcriptomics data from the E-MTAB-8410 study available at the Single Cell Expression Atlas (EMBL-EBI, Wellcome Genome Campus). This dataset includes cells obtained from CRC samples collected from nine individual patients. We excluded cells from the surgical margin (normal-adjacent cells) and all non-tumor cells from the microenvironment. We clustered epithelial (n=4,693) and non-classified (n=174,084) cells into 22 subpopulations and built overall scores to represent EMT and stemness-related features in these cells by using expression levels of literature-based genes associated with these processes in CRC. In addition, we evaluated scores built with gene sets corresponding to the consensus molecular subtypes (CMS) 1-4. Through these evaluations, we determined four clusters (out of 22) highly associated with the EMT (CMS4) profile. Later, we evaluated differentially expressed genes (DEGs) in these EMT-putative cells. Among the top 20 DEGs, only one gene (VIM) has previously been described as an EMT-marker (literature-based gene list and CSM4). In addition, tissue development, cell differentiation, and signaling pathways were enriched using the complete list of overexpressed genes in the EMT-putative cell cluster. Trayectory inference and gene expression across pseudotime of top 20 DEGs were demonstrated. Finally, we evaluated the individual gene contribution of the top 20 DEGs to the prognosis estimation in a CRC bulk dataset. Higher levels of IGFBP7 and TIMP1 were associated ($p<0.05$, log-rank test) with poor prognosis in the Colon Adenocarcinoma (COAD) cohort of The Cancer Genome Atlas (TCGA), whereas HSPB1 and CD63 were confirmed as prognostic factors ($p<0.05$, log-ranktest) in the Rectal Adenocarcinoma cohort (READ-TCGA). With these results, we confirmed that a single-cell approach can be used for evaluating novel strategies to propose EMT-related prognosis biomarkers and therapeutic targets in CRC patients.

Longitudinal Immune Analysis Differentiates Responder And Non-Responder Profiles In Nsclc Patients Treated With Pembrolizumab And Chemotherapy

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Lung cancer is the most diagnosed cancer worldwide and has a poor survival rate, with the majority of cases classified as non-small cell lung cancer (NSCLC). Treatment includes surgery, chemotherapy, radiotherapy, immunotherapy. Despite advances, responses to immune checkpoint inhibitors remain below expectations. This study aims to compare immune profiles between responders (R) and non-responders (NR), evaluated by the iRECIST test at 6 months, to immunotherapy. NSCLC patients treated with Pembrolizumab (anti-PD-1) + Pemetrexed/Platinum (n=33; 16 R and 17 NR) were enrolled. Peripheral blood samples were collected at baseline (T0), 3 weeks (T1), and 9 weeks (T2) post-treatment. Milliplex assays (32 analytes) were performed on plasma samples, while whole blood was evaluated by flow cytometry. Single cell RNA sequencing was performed on 12 baseline samples (6 per group). At T0, NR patients exhibited higher proportions of FGF2, IL-8, IL-10, MIP-1B, and double-positive T cells expressing CTLA-4. At T1, NR patients showed elevated levels of FGF2, IFN-a2, IL-6, IL-8, IL-13, IL-17A, and TNF-a. At T2, NR patients had increased levels of FGF2, IL-4, IL-8, and IFN-a2, while R patients showed higher levels of IL18, CD38highcells, plasmacells, monocytes, classical monocytes expressing HLA-DR, and regulatory B cells (Bregs). These findings align with preliminary single cell sequencing data that revealed differences in monocyte populations, indicating that responders exhibit enriched pathways related to myeloid lineage activation and differentiation. This study highlights the significance of monocytes and their relationship with innate immune responses in the responder environment. It appears that non-responders attempt to structure an immune response but are unsuccessful, with enriched pathways tending toward antigen presentation to T lymphocytes, while responders better structure a response leaning towards an innate immune response. This study highlights significant differences in immune profiles between responders and non-responders to immunotherapy, offering insights that could help future personalized treatment strategies.

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Single Cell LATAM Symposium: Connecting Communities and Advancing Research 2024

Abstracts selected for poster presentations

Platform For Personalized Therapeutic Testing In Breast Tumors

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Breast cancer is the second most common cancer worldwide, with over 2.3 million new cases and 670,000 deaths globally in 2022. In Brazil, treatment costs range from R$ 11,000 to R$ 93,000/per case, depending on the disease stage in post-menopausal women. Understanding the disease and proposing more effective first-line chemotherapy treatments can reduce treatment time and costs, improving patients' quality of life. Targeted therapies that exploit dependence on specific oncogenes or synthetic lethality have been developed, but cancer's heterogeneity drives primary or acquired resistance to treatments. Successful treatment protocols, therefore, must consider molecular stratification and intratumoral heterogeneity. Patient-derived tumor xenografts (PDTXs) and patient derived tumor cells (PDTCs) have emerged as powerful preclinical models that recapitulate tumor diversity, sharing molecular and architectural characteristics with their tumors of origin. PDTXs and PDTCs hold promise for improving success rates in developing new chemotherapeutics and treatment protocols. This project proposes adding breast cancer samples from a Brazilian cohort to LNBio's biorepository and subsequently to the institution's biobank. These samples will provide a powerful resource for pharmacogenomic and preclinical breast cancer studies, including biomarker identification for response or resistance and serving as a platform for chemotherapy testing. PDTCs will be evaluated in 3D growth as organoids to better emulate the tumor microenvironment. Patient tumors, PDTXs, and PDTCs in the biorepository will be assessed using single-nuclei RNA sequencing, spatial transcriptomics, exome sequencing, metabolomics, and proteomics. Data will be compared with individual tumors, PDTXs, and PDTCs to verify consistency. Finally, organoids will be evaluated for their response to current chemotherapies used in the Brazilian public health system (SUS) and tested with new compounds. The obtained data will be compared with clinical outcomes of patients undergoing the same chemotherapies. This project aims to improve the understanding of tumor heterogeneity, enhance biomarker discovery, and develop more effective cancer treatment protocols.

Screening Of Enriched Macrophage Subpopulations In Ulcerative Colitis Using Single-Cell Data

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Macrophages play an important role in normal homeostasis and during various pathological conditions such as infection, inflammation, and cancer. The specialized functions of macrophage subsets are reflected in the molecular tools they express, such as scavenger receptors, adhesion molecules, and receptors for soluble mediators such as cytokines, chemokines, and growth factors. Nevertheless, there is no full knowledge about these macrophage subpopulations using bulk datasets. In the context of gastrointestinal conditions, ulcerative colitis (UC) is a chronic inflammatory disease that often precedes the development of colorectal cancer (CRC). Identifying macrophage subpopulations involved in the chronic inflammatory processes of UC (then exacerbated in CRC) could enhance screening programs and therapeutic strategies. In this study, we aim to use single-cell datasets to evaluate macrophage subpopulations in the contexts of UC and CRC. Herein, we analyzed the cohort GSE162335 composed of single-cell CD45+ cell collections of uninflamed (16 878 cells), pouchitis (20 678 cells), and UC (18 375 cells) samples. We observed the primary classification of cells and observed that UC samples have more monocytes/macrophages (7.89%) than pouchitis (6.40%) and uninflamed (1.14%) samples. Then, we decided to focus on this subset of cells and evaluated the expression of conventional gene markers for M1 (CD68, CD86, SIGLEC1, TLR2, TLR4) and M2 (CD163, MRC1, MSR1) macrophages. After applying Uniform Manifold Approximation and Projection (UMAP) to cluster this group of cells, we observed that UC- related clusters expressed more TLR2 and CD163 levels than healthy samples. Based on these observations, we further evaluated genes overexpressed in UC clusters and find 53 relevant regions (Fold change>2) including CXCL1, DUSP4, IL6, IL10, and MMP19 genes. For further steps, we aim to evaluate levels of these pre-selected genes in a CRC single-cell dataset (E-MTAB-8410) to evaluate if specific macrophage subpopulations are enriched in the path of CRC patients.

AUSTRAL-Omics: A Core Research Facility To Establish And Expand Multi-Omics Technologies Including Single Cell Genomics In Chile

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Single-cell genomics offers unprecedented insights into cellular heterogeneity. In South America, and particularly in Chile, the potential of Single-cell approaches is vast. The region's rich biodiversity and unique ecosystems stand to benefit greatly. In biomedicine, this technology can aid in identifying new therapeutic targets and personalizing treatments, crucial for a genetically diverse continent. It also offers better understanding and management of prevalent regional diseases. Environmentally, single-cell genomics can be utilized to study species adaptation and evolution in response to climate changes and other environmental factors, essential for biodiversity conservation. For instance, analyzing cells from organisms in extreme environments like the Atacama Desert or Chilean Antarctica can uncover unique survival mechanisms. In agriculture, single-cell genomics can enhance crop improvement by identifying genetic traits for disease resistance and environmental tolerance. However, South America faces challenges in implementing this technology, including high costs, limited infrastructure, and a shortage of human resources with training and proficiency in laboratory protocols and competence in computational biology for analyzing and interpreting single-cell experiments. In AUSTRAL-omics, a Core Facility of Genomics and Bioinformatics at Universidad Austral de Chile, we work to overcome some of those challenges. Over the past decade, It has become a leading sequencing lab in Chile serving academia, research centers, public institutions, and private sector with advanced sequencing technologies, including Illumina, MGI, and Oxford Nanopore platforms. Located in southern Chile, AUSTRAL-omics has significantly contributed to the decentralization of knowledge and the strengthening of science and technology in the region. As an advanced sequencing facility with robust bioinformatics resources and skilled personnel, AUSTRAL-omics aims to offer single-cell short-read (Illumina and MGI) and long-read (Oxford Nanopore) sequencing across biomedical, environmental, and agricultural research. The application of these techniques promises to be a powerful tool for exploring Chile's biodiversity and foster national scientific advancement and international competitiveness.

A Human Cellular Map Of The Diversity Of Latin America: Experience In Uruguay"

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The Human Cell Map of Latin American Diversity aims to create a comprehensive cellular atlas of immune cells from diverse populations across Latin America. The project seeks to investigate gene expression patterns in immune cells and map variations in indigenous and mixed-ancestry populations, addressing the underrepresentation of these populations in biomedical research. This can reveal unique gene expression patterns crucial for precision medicine. Uruguay's participation in this initiative focuses on the single-cell RNA sequencing (scRNA-seq) of immune cells to contribute to the map of Latin America. A total of 24 samples from Uruguay will be included, to characterize all immune cell subtypes, including rare populations such as dendritic cells. Blood samples were collected from individuals with mixed ancestry, which will be processed for scRNA-seq using the Chromium platform, and genotyped via microarrays covering 1.5 million positions to estimate ancestry. In addition to sequencing, the project will include a detailed bioinformatics analysis, involving quality control, normalization, clustering, and differential expression analysis of the single-cell data. This comprehensive approach aims to identify cell-type-specific gene expression patterns and elucidate molecular markers pertinent to the immune response in mixed-ancestry populations. The progress of the project and a genotypic characterization of the Uruguayan population will be presented, with the aim to demonstrate the presence of genetic admixture in our population. Additionally, a proposal for future analyses will be outlined to further explore the findings and their implications. Uruguay's contribution is vital in ensuring diverse representation within the Human Cell Map, offering insights into the genetic and cellular diversity of Latin American populations.

Comparative Transcriptional Profiling of BALB/c and C57BL/6 Mice Using Single-Cell RNA Sequencing

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Leishmaniasis is a disease caused by parasites of the Leishmania genus. Leishmania parasites exhibit a digenetic life cycle, with an extracellular promastigote form that lives in the sandfly vector and an intracellular amastigote that replicates within macrophages of mammalian hosts. Upon phagocytosis, Leishmania parasites develop within phagolysosome-derived structures known as parasitophorous vacuoles, where they delay cytotoxic responses in order to grow and replicate. Single-cell RNA sequencing (scRNA-seq) offers a detailed view into the complex transcriptomic dynamics of individual cells, making it ideal for understanding immune responses to infections. This project aims to use scRNA-seq to analyze the transcriptomes of murine peritoneal cells during infection with Leishmania panamensis, comparing responses in two mouse strains, BALB/c and C57BL/6. These strains are known to exhibit divergent immune responses to Leishmania infections, providing a model to characterize molecular determinants of susceptibility and resistance. Peritoneal cells will be isolated from BALB/c and C57BL/6 mice, infected with L. panamensis, and harvested at 3, 6, 12 and 24 hours post-infection to capture the dynamic changes in gene expression. The scRNA-seq data will enable the identification of distinct cell populations within the peritoneal lavage and their transcriptional responses, providing insights into the cellular mechanisms driving infection outcomes in each strain. Futhermore, in vivo infection models will be employed to validate findings and to understand how systemic interactions and the tissue microenvironment influence transcriptional profiles. By comparing in vitro and in vivo data, we aim to identify strain-specific transcriptional signatures and immune cell populations that correlate with susceptibility and resistance to L. panamensis infection. We expect to identify differentially expressed genes involved in cytokine signaling, antigen presentation, and cell migration. These findings will deepen our understanding of host-pathogen interactions in L.panamensis infections and allow the identification of potential therapeutic targets and biomarkers for disease susceptibility and resistance.

Exploring Genomic And Gene Regulatory Variation Across Latam Through Advanced Methods Insingle-Cell Genomics

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Understanding the genetic basis of adaptive phenotypic variation in humans has been a long goal in evolutionary biology. However, genomic studies have been focused mainly on European ancestries, missing most of the remarkable genetic diversity in Latin American populations. In this project, we are profiling both genetic and regulatory variation at the single-cell level across indigenous communities from seven LATAM countries, including 650 individuals. We will mainly focus on profiling the immune system, as it is known for showing wide adaptive variation across populations in response to a history of pathogen infections, diet and environmental exposure. To make this possible, we have optimized a new protocol for on-site isolation of peripheral blood mononuclear cells (PBMCs), eliminating the need for specialized equipment when sampling on remote locations. In a preliminary pilot study, we successfully applied this method to 42 individuals from México and Colombia, representing Otomí, Mayan, piapoco and wayú ancestries. High quality single cell transcriptomes were obtained showing enhanced cell type annotation, and population specific expression signatures. Notably, by studying immune response variations at the population level, we expect to inform about ethnic-specific disease prevalence as well as molecular markers for diagnosis and prognosis.

SUPML: A Novel Algorithm For Predicting Surface Markers In Single-Cell Data

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Single-cell RNA sequencing (scRNA-seq) enhances the study of cell interactions, gene regulation, and cellular dynamics, improving our understanding of cellular heterogeneity and identifying new cell populations. A current challenge is experimentally validating in silico subpopulations by finding reliable cell type-specific or state-specific surface markers, crucial for purifying particular cell types for research and clinical applications. Here, we applied the LightGBM algorithm, a machine learning method, to identify surface markers specific to each predicted subpopulation. To our knowledge, no tool is capable of selecting a robust set of surface markers to assist in the generation of antibody panels. We have used as input integrated scRNA-seq data from a previous study from our group consisting of 13 public datasets from 7 different cancer types that aimed to identify tumor-associated myeloid cells. ScRNA-seq gene sets representing different subpopulations were filtered for surface coding genes (SCG) according to the Cell Surface Protein Atlas (CSPA) database. The expression of SCGs for each subpopulation was standardized using the z-score. The LightGBM model was trained on 70% of the data (training set) and tested on the remaining 30% (test set). A 30-fold cross-validation was used to adjust the hyperparameters. To evaluate the performance of our model, we analyzed accuracy, sensitivity, specificity, and AUC. Additionally, we calculated the Shapley values of each SCG according to the predictive model to understand their respective contributions using SHAP (SHapley Additive exPlanations). Training the LightGBM model on scRNA-seq data from macrophage signatures RTM_Int and Mac_Hypo yielded an AUC of 0.98, an F1-score of 0.81, and an accuracy of 0.91. Furthermore, by examining SHAP values, we identified STAB1 and SLC2A1 as potential unique markers. In conclusion, we demonstrated that our machine learning algorithm, based on surface gene expression, robustly selects potential markers to facilitate in vitro and screening experiments by enabling the selection of specific antibodies.

Single Cell Analyses and Stromal Differentiation and Its Spatio-Temporal Relationship with the Developing Prostatic Epithelium

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The prostate is the focus of various diseases, including prostatitis, benign prostatic hyperplasia, and prostate cancer. Prostate cancer will affect 12.5% of men of all ages and 50% of men at the age of 85. Benign prostatic hyperplasia affects a larger number of people and occurs earlier. Our interest lies in understanding prostate physiology from a cellular and molecular biology perspective to better comprehend the behavior of this organ and its different cell types in diseases. Our project is related to the aspects of prostate development, focusing on the cellular interactions that occur at the tip of epithelial growth, particularly the relationship between the differentiation of smooth muscle cells and the settlement of p63+ cells along the epithelial structure, considering metalloproteinases. Much has been discussed about the epithelium-stroma interactions in development, prostate function, and disease establishment. Several pairs of signal-ligands have been identified as participants in this interaction, with one compartment expressing the ligand and the other expressing the cognate receptor. However, minimal attention has been given to the epithelial growth front and its relationship with smooth muscle cell differentiation in the prostate. We have already identified a marked division in the differentiation front of smooth muscle cells concerning the epithelial growth tip. In this project, we aim to establish the role of different cell types and their interactions, as well as identify the involved molecules, always seeking to characterize the responsible mechanisms. Besides epithelial and stromal cell subtypes, we will be committed to identify immune system cells, especially macrophages, that participate in different processes, particularly in response to castration and experimental autoimmune prostatitis. More broadly, we intend to employ single-cell analyses to identify stromal differentiation sequences in prostate development.

Evaluation of Cellular Immune Response in Rheumatic Heart Disease: Unveiling the Functional Profile of T Lymphocyte Subpopulations Through Single-Cell RNA-seq and Flow Cytometry.

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Rheumatic Heart Disease (RHD), predominantly found in low-income countries, is caused by bacterial infection with Streptococcus pyogenes. The pathogenesis of RHD involves antigenic mimicry between bacterial and valvular proteins, which triggers a persistent immune response in the heart valves. The elevated presence of infiltrating T cells in the valvular tissue, which secrete inflammatory cytokines, combined with the reduction of regulatory cytokine-producing cells, may contribute to the maintenance of valvular tissue damage in patients with RHD. The mechanisms of cellular recruitment to the valvular tissue are not yet fully understood. The binding of autoreactive antibodies to cardiac proteins and bacterial antigens in the valvular endothelium may facilitate T-cell infiltration by increasing VCAM1 expression. Considering that T lymphocytes are the main cellular subpopulations involved in the adaptive immune response in chronic diseases and their possible association with the immunopathogenic response in RHD, we aim to characterize the functional profile of different peripheral T lymphocyte subpopulations in individuals affected by this disease. Our study includes the transcriptional evaluation of T lymphocyte subpopulations through single-cell RNA-seq analysis, which allows for a high- resolution view of gene expression at the individual cell level, providing insights into the heterogeneity and functional states of these cells. Additionally, we will investigate the functional profile of CD4+, CD8+, and DN (CD4-, CD8-) cells through the expression of cytokines and their receptors, cytotoxic molecules, and chemokine receptors using multiparametric flow cytometry. Specific knowledge of the cellular sources of inflammatory and regulatory cytokines, as well as molecules potentially involved in valvular tissue damage, is crucial for understanding the mechanisms related to the pathogenic immune response in patients with RHD. This understanding is fundamental for the identification of targeted immunotherapeutic strategies that may prevent disease progression and valvular damage.

Investigating Influenza Vaccine Response At The Single-Cell Level

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During the Covid-19 pandemic, mRNA vaccines came into the spotlight as an alternative immunization method, capable of comparable and sometimes better results than more traditional vaccination strategies. This caused a renewed interest in the manufacturing and testing of mRNA vaccines for other diseases, like influenza in the case of this project. Single-cell mRNA sequencing allows for the examination of transcriptional patterns in a more detailed level, making analysis at cell type level possible, instead of the more traditional tissue-level done with bulk mRNA sequencing. In this project, seven patients were vaccinated with two different vaccination technologies (four with mRNA and three with the commercially available vaccine) and had lymph node and blood samples collected in different timepoints, up until six months after vaccination. The blood samples were enriched for B cells, and all samples were used to generate single-cell mRNA sequencing data. With this data in hand, we have searched for differences in immune response between the two vaccination groups, aiming to see if the mRNA vaccine was performing as expected. After the identification of B cell types, experimental examination has shown that there is evidence for a permanence of immune response after six months for two patients, both inoculated with mRNA vaccines. Considering these latest results, we are now focused on understanding the genes and molecular pathways that could be the drivers responsible for the long term immune response in these two cases by comparing them to the other patients. In summary, we are examining the immune response to traditional and mRNA vaccination for influenza in a single-cell level, and hope to shed more light into the genes involved in long term immune response after immunization.

Single-Cell Alternative Splicing analysis in Ovarian Cancer Microenvironment

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Ovarian cancer is the most deadly type of gynecological cancer. Among ovarian cancer subtypes, High-grade serous ovarian carcinoma (HGSOC) is distinguished by its high rates of treatment resistance and mortality. The alternative splicing (AS) increases the diversity of the transcriptome and proteome by allowing exons and introns from the same pre-RNA to be deleted or maintained. AS is essential for crucial biological processes such as cell differentiation, and its dysregulation has been related to a variety of diseases, including cancer, by causing the expression of isoforms that promote tumor growth. Thus, single-cell isoform identification can reveal distinct splicing patterns associated with aggressive tumor features or a poor prognosis in malignant cells. In this work, we want to use publicly available single-cell RNA sequencing (scRNA-Seq) data from HGSOC patients to discover AS occurrences and particular isoforms associated with therapy response. The 13 scRNA-Seq data was downloaded from the NCBI database using the Fastq Dump tool, and reads were aligned to the reference genome using CellRanger, with Seurat R we identified approximately 96,793 Cells. BAM files were used as inputs for STARsolo to generate the gene expression and splice junction count matrices, for each sample. Afterwards, SingCellaR R was used to prepare the count matrices, and then the Marvel R package was used to perform the clustering, differential gene expression and alternative splicing analysis, and functional enrichment analysis. To identify isoforms and/or splice junctions associated with treatment response, a differential transcript analysis will be carried out. We believe that this work will help us understand the regulatory mechanisms of gene expression mediated by the process of AS, and to enhance our knowledge of the biology of HGSOC.

Characterization of the Transcriptional Profile of Macrophage Subpopulations Altered by Aging and Their Association with Breast Cancer

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Aging is an inexorable process affecting organisms, characterized by chronic low-grade inflammation, a significant risk factor for various diseases, including cancer. Macrophages, key components of the innate immune system, play a pivotal role in these changes, acting as sentinels responding to pathogens and tissue damage, thereby triggering inflammatory responses. This highlights the intricate interplay between aging, inflammation, and cancer. In breast cancer, the most prevalent cancer in women worldwide, with incidence increasing in older women, understanding how macrophage subpopulations adapt to aging could reveal mechanisms promoting tumorigenesis and influencing clinical outcomes. Using single-cell transcriptomic data from breast cancer 105 patients and 73 healthy donors, we have integrated 697,677 high-quality cells using scVI and scANVI algorithms. The latent space was projected into 2D using UMAP, with clustering performed using the Leiden algorithm and manual annotation based on canonical markers and differentially expressed genes via the Model-based Analysis of Single-cell Transcriptomics algorithm. We identified 14 main cell types, further subgrouped into 46 cell subpopulations/states across different molecular subtypes of breast cancer and healthy tissue. Among macrophages, five subpopulations were identified, notably Mac_AgPress, which showed increased abundance in older donors but decreased antigen presentation and phagocytosis capabilities with age. Deconvolution in the breast cancer bulk RNA-Seq cohort from The Cancer Genome Atlas using BayesPrism showed that Mac_AgPress enrichment was associated with better survival in Triple-Negative subtype patients. Our findings provide insight into the cellular phenotypes and their roles in the TME of breast cancer, particularly regarding macrophages and their correlation with aging, which may contribute to developing new treatment strategies, and provide a more comprehensive understanding of the tumor microenvironment in breast cancer.

A Single-Cell Atlas For Traumatic Brain Injury: Brain Cell Specific Transcriptional Alterations As A Result Of Head Trauma

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Traumatic brain injury (TBI) is a prevalent condition worldwide, often resulting from various types of blunt-force impacts to the head region. TBI can lead to long-term debilitating outcomes, including neuropsychiatric diseases, such as schizophrenia and mood disorders, as well as other neurological conditions, such as post-traumatic epilepsy. Post-injury processes, including inflammation and rearrangement of neuronal networks, involve transcriptional alterations of several different brain cell types, such glial cells, endothelial cells, and many neuronal subtypes. The use of single-cell technologies to investigate the gene expression in TBI is thus particularly interesting, as it facilitates the identification of cell-type specific pathobiological changes, as well as brain cell adaptive responses to TBI. The aim of this project is to develop a TBI cell atlas, consisting of single-cell and single-nucleus RNA-seq datasets that include control and post-TBI brain samples. Systematic search of Gene Expression Omnibus and BioProject NCBI databases has resulted in 17 mouse and one human TBI dataset, containing over 140 samples and 850.000 cells, collected from different brain areas, such as cortex and hippocampus. TBI sources include controlled cortical impact and mild repetitive brain injury in mouse models, and severe, acute TBI in humans. Quality control was performed to exclude cells containing outlier gene expression of nuclear and mitochondrial genes. We are currently testing Seurat and scvi-tools single-cell integration and clusterization approaches for these different datasets, to minimize batch effects. After this step, we will analyze differential gene expression in cell-type specific clusters, followed by Gene Set Enrichment Analysis and Over-representation analysis, to identify enriched pathways and biological processes in the different brain cells. These analyses can further elucidate changes in cellular and molecular networks that occur in the brain following TBI, which can aid in understanding the etiology and suggest molecular targets for the prevention of TBI-related outcomes.

Project Jaguar: Mapping immune cell diversity across Latin America

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The immune system has a wonderful plasticity, which allows it to adapt and protect us against pathogens and autoimmune diseases. Genomic diversity behind immune system is influenced by genetic and environmental factors, leading to variable immune responses across regions. Currently, immunogenomics studies have mainly focused on European populations, leaving others underrepresented, as Latin Americans (LATAM). LATAM populations are the result of genetic admixture among European, Indigenous, African and Asian populations, shaped by the rich geographic diversity and historical migrations. Project JAGUAR (Joining All: Genes, immUnity And diveRsity) aims to explore how regional genetic diversity interacts with environmental factors to shape immune cells, being the first coordinated effort to expand LATAM participation in the Human Cell Atlas Initiative. The project will achieve its aim through the creation of an atlas of peripheral blood mononuclear cells (PBMCs) in healthy individuals from seven LATAM countries: Argentina, Brazil, Chile, Colombia, Mexico, Peru and Uruguay. Sampling is being done by experts in each country. Sample processing and data generation will be based at the Sanger Institute, using various single-cell technologies such as: scATAC-seq, scRNA-seq and CITE-seq. Analyses will be co-led by centers in the UK, Mexico, Brazil, Chile, and Colombia, with contributions in immunology expertise from Argentina, Uruguay, and Peru. The resulting atlas across LATAM ancestries will help characterize PBMC diversity, generate quantitative information for protein markers and elucidate regulatory networks to map regulations influenced by genetic variation. Altogether, it will be possible to understand the genetic and environmental influences on immune system diversity and contribute to increasing the experimental and analytical capabilities in single-cell genomics at local levels in LATAM. Finally, the atlas will provide a reference for future studies and support the development of precision immunodiagnostics and therapeutics.

Exploring Ancestry Diversity in Immune Cell Profiles through scRNA-seq in Admixed Populations from Brazil

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Despite advances in precision medicine through RNA sequencing, traditional methods often obscure gene expression heterogeneity among cells. Single-cell RNA sequencing (scRNA-seq) allows researchers to dissect this variability and reassess disease subtypes. Rich ethnic diversity and genetic admixture between indigenous peoples and other continental populations characterize Latin America. Differences in cell proportions and immune responses among Latin American individuals with varied ancestry may result from exposure to various pathogens and environmental conditions. However, these ancestries are underrepresented in current biomedical research. To address this gap, our study uses scRNA-seq to characterize PBMCs from healthy individuals across indigenous and mixed populations in seven countries (Brazil, Chile, Colombia, Mexico, Peru, Uruguay, and the US). In Brazil, volunteers from the Albert Einstein Hospital Blood Bank provided health and family history data and donated blood samples. Peripheral blood mononuclear cells are isolated by magnetic bead negative selection and prepared for scRNA-seq with the 10x Genomics Chromium equipment. SNP genotyping and ancestry estimation is being carried out for all samples. The data processing workflow begins with Cell Ranger for initial raw data handling, SoupX for environmental RNA contamination removal, scDblFinder for doublet treatment, and Freemuxlet or Demuxlet for demultiplexing. Seurat is used for normalization and dimensionality reduction, Harmony for data integration, and CellTypist for cell type annotation using reference datasets. So far, we have enrolled 76% of the proposed 100 participants. The average age is 34 years, and the male/female ratio is 4:3. These individuals were born across seven different Brazilian states, with their parents and grandparents representing more than 50% of Brazilian territory. According to participant surveys, approximately 47% reported indigenous ancestry in their family. Our ongoing research will provide valuable insights into the genetic and cellular heterogeneity within the diverse Brazilian population, possibly offering a deeper understanding of how genetic background influences immune responses.

A Pan-Cancer Integrated Analysis Of Lncrnas Central To Tumorigenesis

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Long non-coding RNAs (lncRNAs) are molecules exceeding 200 nucleotides in length and not translated into proteins. Their significance in cancer has gained increasing recognition in the literature, as the processing of these molecules is systematically altered in tumors, highlighting their critical role in tumorigenesis, growth, and progression. LncRNAs exhibit remarkable diversity in structures and sequences, posing challenges for bioinformatics-based prediction of their tumor-related functions. One approach often employed in the literature to predict the biological function of these RNAs follows the "guilt-by-association" principle. By utilizing co-expression network analysis, it can be inferred that when the expression of lncRNAs and protein-coding genes with known functions is correlated, they may share similar functions. However, the high variability in gene expression across different tumors presents a significant obstacle for pan-cancer co-expression network analyses. Consequently, ranking is performed based on the correlation between gene pairs, disregarding the network structure. Furthermore, lncRNAs can play crucial regulatory roles, often being cell-, tissue-, or even developmental stage-specific, introducing another challenge for pan-cancer analyses. By refining the methodology, we could reduce false positives and enhance the functional characterization of newly described lncRNAs, associating them with specific cell types and enabling a better understanding of tumor biology. This project aims to conduct a pan-cancer integrated analysis to identify lncRNAs central to various tumor types. Initially, public TCGA RNA-seq data from paired samples (tumor and adjacent tissue) will be utilized for the following types: breast, kidney, prostate, lung, stomach, and uterus. Co-expression networks will be generated for each tumor type using the WGCNA package. A spectral comparison methodology of lncRNA-centered subnetworks will be employed to identify relevant lncRNAs shared across multiple tumors (coga package). Finally, single-cell sequencing data for these tumor types will be retrieved from the GEO database, and new networks will be generated using the hdWGCNA package. This will allow for the comparison of subnetworks found in bulk sequencing data with single-cell networks, enabling the assessment of overlap and how these genes are expressed in different cell types. These subnetworks will be enriched using the enrichR package, providing valuable clues about lncRNA functions. These molecules can be experimentally validated by laboratory collaborators, with emphasis on the potential associated pathways identified. By integrating pan-cancer data with single-cell sequencing, the study aims to identify lncRNAs with critical functions in tumorigenesis. The findings could pave the way for the development of novel diagnostic and therapeutic strategies for cancer.

Single Cell Rna-Seq To Understand Surface Protein Gene Expression Heterogeneity In T. Cruzi Populations

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Trypanosoma cruzi, the causative agent of Chagas disease, poses a significant public health challenge in Central and South America, affecting approximately 8 million people with millions more at risk of infection. This protozoan exhibits unique characteristics, particularly in gene expression, which present distinct challenges in combating the disease. T. cruzi genes are organized into polycistrons, typically transcribed constitutively but showing variable transcripts steady-state levels, indicating post-transcriptional regulation mechanisms. Transcriptomic studies across its life cycle have revealed differential expression patterns that underscore its complex gene regulation mechanisms. The life cycle of T. cruzi involves transitions between epimastigote, metacyclic trypomastigote, amastigote, and blood trypomastigote stages, each characterized by significant morphological and molecular changes adapted to different hosts and environments. Unlike other trypanosomatids, T. cruzi lacks antigenic variation mechanisms but relies on diverse surface proteins encoded by large multigene families, including trans-sialidases, Mucins, MASP, and GP63, crucial for infectivity and immune evasion. Despite extensive research on population-level expression profiles, intra-population variability in surface protein expression remains poorly understood. Recent advancements in single-cell RNA sequencing offer a promising avenue to uncover subtle molecular mechanisms relevant to pathogenesis, as demonstrated in related parasites like P. falciparum and T. brucei. Our research aims to elucidate transcriptomic heterogeneity within T. cruzi parasites from the trypomastigote form using single-cell RNA-seq data. By focusing on surface protein gene expression diversity, this research seeks to deepen our understanding of T. cruzi's cellular biology and infection strategies. The insights gained in this study could inform future therapeutic strategies targeting virulence mechanisms of this medically important parasite.

Interaction of Lipid-Associated Macrophages in the Tumor Microenvironment of Breast Cancer Using Single-Cell Sequencing Data

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Background: Breast cancer (BC) is the most common neoplasm in the world among females, with over 2.2 million new cases annually. Despite advances in new therapies and early diagnostic techniques, BC still accounts for 15% of all cancer deaths in women each year, approximately 680,000 globally. Solid tumors like BC have a tumor microenvironment (TME) extremely rich in immune cells, among which macrophages are increasingly gaining attention due to the role these highly plastic cells play in the clinical outcome of patients. Among the phenotypes that macrophages can be associated with, a recent study from our group demonstrated that lipid-associated macrophages (LA_Macs), characterized by the expression of TREM2 and enriched for pathways related to extracellular matrix remodeling and amino acid metabolism, are important prognostic biomarkers in patients with triple-negative BC. Main Goal: The project aims to utilize public scRNA-seq data to elucidate key cell-cell communication pathways between LA_Macs and other cells in the TME that can impact patient prognosis and response to therapy. **Methodology:** All data will be downloaded from public databases such as the Gene Expression Omnibus following the exclusion criteria: samples that are not from human biopsies and that do not contain immune cells. The data will then go through quality control steps and will be integrated using scVI after detecting highly variable genes with Scanpy, which will be used to handle the data throughout the project. Cell communication analysis will be performed using Cellchat. Preliminary **Results:** Initially, three datasets were downloaded (Shiao et al, Bassez et al, and Kumar et al), totaling 888,294 cells and 118 patients who received or not any kind of treatment such as chemotherapy, radiotherapy and anti-PD1, and being in different stages (I, II, III) of the disease. After the quality control step, 838,000 cells were recovered.

Applying Single-Cell Long-Read Sequencing to Explore Mitochondrial Genomes in Toxoplasma gondii and Related Parasites

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Mitochondria are crucial organelles in eukaryotic cells and key therapeutic targets against apicomplexans. Despite their importance, the genomic structures of atypical mitochondria in organisms like apicomplexans and dinoflagellates are poorly understood. The Toxoplasma gondii mitochondrial genome, for instance, exhibits complex heterogeneity, comprising variable-sized fragments that rearrange into different configurations. This complexity is also seen in dinoflagellates, complicating our understanding of genetic and functional diversity within these populations. Current genomic methodologies fail to capture the intracellular variability and dynamics of mitochondrial genomes, especially in non-traditional organizations that resist standard reconstruction techniques. Recent advances, however, have enabled genomic sequencing of bacterial single cells and unicellular eukaryotes, as well as mammalian mitochondrial genomes, opening avenues for exploring population heterogeneity at specific loci or across entire genomes. Our research aims to apply single-cell mitochondrial genome sequencing, inspired by recent bacterial and human mitochondrial studies. These techniques involve isolation of mitochondria and/or amplification of mitochondrial DNA using short and long read sequencing technologies, thus we aim to minimize the amplification of nuclear DNA, allowing a more accurate characterization of the mitochondrial genome. We plan to use this methodology initially on T. gondii and related parasites like Neospora caninum. This will enable us to resolve the mitochondrial genome configuration of each parasite, identify the presence of heteroplasmy, and determine the arrangements of the three genes present in the mitochondria. Subsequently, we will apply this approach to more distant species, such as dinoflagellates. This innovative approach could improve our understanding of mitochondrial dynamics in pathogenic eukaryotes, potentially paving the way for targeted genetic studies and therapeutic advances.

Single-Cell Rna Sequencing Applied To The Elucidation Of The Role Of Nkg2d During The Interplay Between Natural Killer Cells And Myeloid-Derived Suppressor Cells In Triple-Negative Breast Cancer

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Myeloid-derived suppressor cells (MDSC) from spleen and the tumor microenvironment (TME) play a critical role in tumor progression and resistance to immunotherapy. Natural killer (NK) cells become activated upon engagement of receptors such as NKG2D, which recognizes ligands predominantly expressed on tumor cells, such as MICA (MHC class I-related protein A). Although MDSC can negatively affect NK cell activity against tumor cells, little is known about the effect of activated NK cells on MDSC. Accordingly, we aim to investigate the interplay between activated NK cells (that sensed activation stimuli mostly through NKG2D) and MDSC using single-cell RNA sequencing (scRNA-seq) technology. Preliminary results demonstrated that subcutaneous injection of 30,000 4T1 cells, a murine highly metastatic TNBC cell line, induced aggressively growing tumors, lung metastases, and splenomegaly in BALB/c mice. However, mice challenged with 4T1 cells engineered to express MICA (4T1-MICA) required doses of 250,000 cells to achieve sustained tumor growth. Notably, 4T1-MICA tumors exhibited a biphasic growth characterized by an early regression followed by a resumed growth after day 15, without the splenomegaly characteristic of 4T1 tumor-bearing mice. The growth of 4T1-MICA tumors was associated with a reduced frequency of splenic MDSC, enhanced NK cell function, and fewer lung metastases. We conclude that expression of MICA on 4T1 tumor cells promotes sufficient engagement of NKG2D and NK cell activation that limits MDSC expansion and metastasis. We plan to in-depth characterize the expression of immune mediators in intratumoral and splenic NK cells and MDSC from 4T1- vs 4T1-MICA-bearing mice through scRNA-seq. We aim to unravel the transcriptomic profiles of NK cells (activated through NKG2D) and MDSC, to better understand the underlying mechanisms that lead to a biphasic tumor growth and reduced metastases in our experimental model. These insights could enlighten targeted immunotherapeutic strategies to reshape the TME, potentially enhancing treatment efficacy.

Single-Cell Rna Sequencing Applied To The Elucidation Of The Effect Of A Senescent Environment On The Quality Of The Immune Response Against Tumors

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Aging is associated with immunosenescence, a phenomenon that involves immune cell anomalies that limit their response to infections, vaccines and neoplastic cells. Senescent cells accumulate in tissues over time, contributing to a pro-tumorigenic microenvironment through the senescence-associated secretory phenotype (SASP), which includes cytokines, chemokines, and proteases. However, the effect of such accumulation on the immune system remains ill-defined. We observed that senescent fibroblasts (SenFb) inhibit IFN-γ production by natural killer (NK) cells and affect macrophage polarization, increasing the expression of M2-associated markers (CD206, MARCO) and decreasing the expression of M1-associated markers (CD86, CD274), compared to control fibroblasts (ConFb). In mice, co-injection of SenFb with CT26 cells resulted in faster tumor growth compared to ConFb. Tumors from the SenFb group exhibited higher proportions of CD45+ cells but reduced percentages of NK cells with lower PD-L1 expression. Spleens of these mice presented higher proportions of NK cells with reduced NKG2D and TIM-3 expression, and increased PD-1 expression. Therefore, SenFb may promote an immunosuppressive pro-tumorigenic environment that impacts on NK cell functionality and macrophage polarization. As multicolor flow cytometry has certain limitations in assessing the tumor microenvironment (TME), using Single-Cell RNA sequencing, we propose to in-depth characterize the expression of immune mediators in intratumoral and splenic NK cells and macrophages from mice challenged with SenFb+CT26 tumor cells vs ConFb+CT26 tumor cells to dissect transcriptomic profiles shaped by a senescent environment and its consequences on the TME at a single-cell level. We aim to identify key signatures and receptors that may drive a dysfunctional immune response and facilitate tumor progression in a senescent context. This approach will provide insights into the causes of a higher incidence of tumors in the elderly and potentially identify therapeutic targets for developing novel immunotherapeutic approaches to enhance anti-tumor immunity and improve cancer treatment.

Understanding the Trajectory of Differentiating Monocytes in the Inflammatory Process of Atherosclerosis and in a LATAM Healthy Population

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Cardiovascular diseases (CVD) are the leading cause of death worldwide and often result from advanced atherosclerosis, a chronic inflammatory condition. Monocytes are essential in the initiation and development of atheroma plaques due to their ability to adhere to vascular walls, transform into macrophages, and accumulate lipids, eventually leading to the formation of atherosclerotic plaques. In this process, different chemokines, adhesion molecules, and receptors such as CD36 and Low- density Lipoprotein Receptor-related Protein 1 (LRP1) are involved. CD36 and LRP1 are responsible for the endocytosis of modified LDLs, a crucial factor in the disease's progression. Given the importance of these receptors as modulators of key signaling pathways in immune and inflammatory responses, this study focuses on characterizing the expression levels of CD36, LRP1, and other genetic factors in monocytes, and studying their differentiation trajectory. Our hypothesis is that there are specific molecular signatures in the transitional states of the differentiation pathway of monocytes in healthy individuals, which predict their susceptibility to acquire proatherogenic phenotypes and the development of atherosclerosis. We propose to analyze single-cell data from the Jaguar project (our project in healthy individuals) and compare it with databases containing data from patients with atherosclerosis and atherosclerotic plaques. Additionally, we will compare this data with single-cell data from healthy bone marrow to determine if CD36, LRP1, or other genetic factors are already altered before monocyte differentiation. The pipeline was developed using Seurat and Monocle packages implemented in R Studio. These insights will enhance our understanding of different key factors in monocyte differentiation and its implications in atherosclerosis, potentially guiding the development of targeted therapies for CVD.

Development of a Vaccine against Schistosomiasis Using Modified Vaccine Candidates and a New Third-Generation Adjuvant

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Schistosomiasis affects over 280 million people globally, causing chronic morbidity and significant socioeconomic impact in developing regions. Despite its prevalence, there is no effective vaccine for schistosomiasis. Our research aims to deliver two innovative products: a novel vaccine against schistosomiasis and a new third-generation adjuvant. These products will work together to induce a robust and long-term immune response against various Schistosoma species. The vaccine development involves using modified vaccine candidates (Sm14, TSP2, and Sm-p80) structurally altered through in silico approaches, including structural modification and molecular dynamics simulations, to enhance their stability. Following these modifications and validations, the vaccine candidates will be expressed and purified for subsequent animal trials. In the animal trials, single-cell RNA sequencing (scRNA-seq) will be employed to analyze the immune cell subsets in liver, intestine, and spleen samples from vaccinated mice, as well as Schistosoma larval and egg samples. This technology will provide detailed insights into the specific immune responses induced by the vaccine formulation, identifying immune cell subsets and their roles in the response to the parasite. By obtaining this information, we aim to reduce the risk of project interruption. Preliminary studies with collaborators have shown that one of the adjuvant candidates, FK34, in combination with LPS, significantly enhances Th1-type immune responses while reducing IL-10 production, compared to the natural MDP and LPS combination. These findings suggest that the FK34/LPS combination has the potential to be an effective adjuvant in the schistosomiasis vaccine formulation. We expect to demonstrate the efficacy of the MDP-based molecule as an adjuvant, identify the most effective modified antigens, assess the safety profile of the new vaccine formulation, and verify the long-term protection conferred by the vaccine through challenge studies in infected animals. Continued research and funding are necessary to confirm these findings and advance the development of these two products for the effective prevention and control of schistosomiasis.

Phenotypic And Functional Characterization Of Adipose Tissue Lymphocytes Of Patients With Obesity Using Single-Cell Technologies

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Background: Obesity is a growing worldwide concern that affects close to 2 billion people. Although all branches of the immune system play a role in setting the inflammatory tonus during this disease, the role and abundance of cells of the adaptive immune system and innate lymphocytes are not fully uncovered yet. Therefore, we will perform single nuclei RNA-seq and flow cytometry in samples of subcutaneous (SAT) and visceral adipose tissue (VAT) of patients undergoing bariatric surgery. **Proposed methods:** 14 SAT and 15 VAT samples from 15 donors were collected (7 male, 8 female, mean age 42.85±10.51). After the separation of the stromal vascular fraction (SVF), the RNA of nuclei was isolated and libraries were prepared and sequenced. The raw data was processed using Cell Ranger and will be analyzed using the Seurat, SingleCellExperiment and Azimuth packages (R) and integrated with data available in public databases using the scANVI package (Python). The identified clusters will be confirmed and sorted by flow cytometry using commercial antibodies against the selected targets. **Preliminary Results/Expected Results:** Our dataset is currently the second biggest regarding the adipose tissue of patients with obesity. For the clustering of the cells in individual samples, we expect to observe a robust cluster of lymphocytes and their main subtypes, such as the T and NK cells, which will be selected for further analysis. When the data on the integration of lymphoid cells emerge, we expect to observe a more nuanced picture of adipose tissue lymphocytes. We also expect to observe the regulated pathways in lymphocytes and therefore trace the functional role of these cells in obesity. Moreover, we expect to identify the differentially regulated cell clusters and molecules in flow cytometry of SAT, VAT and blood. We also expect to observe their association with the clinical data of the patients.

Differentially Expression Anaylsis Between Circulating Tumor Cells (Ctcs) And Tissue Tumor Cells In Costa Rican Her2 Positive Metastatic Breast Cancer Patients With Combined Therapy Of Trastuzumab, Pertuzumab And Taxanes.

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Introduction: Breast cancer stands as the most diagnosed cancer among women globally, holding the top position in both incidence and mortality rates among women in Costa Rica. Circulating tumor cells (CTCs), epithelial-origin cells from tumor tissue, traverse through peripheral blood and bone marrow, harboring the potential to seed new metastatic sites. This study aims to conduct a comparative analysis between CTCs and tumor cells in tissue, employing differential gene expression analysis. The objective is to identify key genes implicated in the epithelial-mesenchymal transition process, pivotal in the development of new metastatic sites in patients undergoing combined therapy involving trastuzumab, pertuzumab, and taxanes.
Methods: Peripheral venous blood samples will be collected from four patients at intervals of 8 to 12 weeks (totaling of 3 samples per patient) to isolate CTCs based on cell size and markers such as cytokeratin (CK), DAPI and CD45. Single-cell mRNA libraries from CTCs will be constructed using the BD Rhapsody Whole Transcriptome Analysis (WTA) Amplification kit. We will compare this data against breast cancer cell databases to analyze the differentially expressed genes (DEGs) in both cellular environments and track the evolution of CTCs over sample collection intervals. Dimensional reduction techniques such as PCA and UMAP will be utilized to facilitate cellular clustering process and cell type annotation. Cell-Cell Communication Analysis (LIANA) will be employed to infer ligand-receptor interactions of CTCs, identifying overexpressed receptors compared to tumor cells in tissue. Pseudotime analysis (Monocle) of CTCs will infer the evolutionary trajectory of CTCs across different cluster types.
Expected outcomes: the study aims to assess various single-cell states of CTCs and crucial ligand-receptor interactions facilitating adhesion to the endothelium and the generation of new metastatic sites compared to tumor cells in tissue. Specifically, it seeks to demonstrate the evolution of subsets of CTCs with gene expression patterns that may confer a high metastatic capacity through specific ligands in the context of patients receiving targeted therapy with trastuzumab, pertuzumab, and taxanes.

Uncovering The Transcriptomic Heterogeneity Of Immune Cells Involved In The Progression Of Hpv-Negative Head And Neck Squamous Cell In A Single-Cell Perspective

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Head and neck squamous cell carcinoma (HNSCC) is a diverse group of cancers arising from the mucosal surfaces of the head and neck region. The heterogeneity of HNSCC, along with its tumor microenvironment (TME) and the immune cells that compose it, requires technologies such as single cell RNA sequencing (scRNA-seq) to characterize them. The composition of TME cells can influence tumor progression. This work aims to identify markers and cellular interactions by comparing samples with (N+) and without (N0) metastasis. We integrated two previously published scRNA-seq datasets of HNSCC, focusing on the role of immune cells in shaping disease progression. We investigated CD45+ cells in tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) from HPV- samples due to the aggressive nature of these tumors and the presence of advanced disease and metastasis in patient cohorts. Using Cellranger for initial data analysis, Seurat for dimensionality reduction and clustering, along with Celltypist for cell annotation, we found that TIL and PBMC from N+ have different cell proportions compared to N0 samples, particularly regarding NK cells, T cell subtypes and monocytes/macrophages. Furthermore, through differentially expressed genes (DEG) analysis, we demonstrated that Tem/Temra cytotoxic T cells from N+ TILs showed a massive reduction in gene expression compared to N0. Among the downregulated genes were genes such as KLRK1, IKZF2 and FCGR3A, which are crucial in the cytotoxic activity of these cells. To analyze the cellular interactions causing this modulation, we used Cellchat. This analysis showed that Tem/Temra cytotoxic T cells from N+ had a greater increase in interactions with all the other cells analyzed compared to N0. We will perform pseudotime trajectory analysis for Tem/Temra cytotoxic T cells to better understand these differences between pathological stages of HNSCC. We hope this study can shed light on the immune cell landscape in HNSCC metastasis.

FBXO11 and PTPN11 Identified As Potential Shared Biomarkers Between Hormone-Sensitive Breast And Endometrial Cancers Through A Bulk Multi-Omics Integration Approach

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Breast cancer and endometrial cancer are complex diseases that show a high degree of molecular and histological heterogeneity. Luminal A and B breast cancer patients and type I endometrial cancer patients share molecular and cellular mechanisms, as well as causal factors such as hyperestrogenism. This study aimed to identify shared tumor biomarkers between both diseases. 565 breast cancer patients and 348 endometrial cancer patients from The Cancer Genome Atlas platform were selected based on their histological, hormonal, and immunological characteristics. Their genomic, epigenomic and transcriptomic data, obtained at the bulk resolution, was analyzed separately and through an integrative approach using the multi-view learning algorithm Deep Generalized Canonical Correlation Analysis. Biomarkers were identified for each patients' clusters obtained from the different omics profiles. The multi-omics integration strategy allowed the discovery of biomarkers that were not identified in single-type omics data analysis. The characterization of biomarkers revealed that several of these genes are involved in key biological processes for cancer such as cell proliferation, apoptosis, and angiogenesis. Several genes were found to be biomarkers of all 913 patients. Among them, FBXO11 and PTPN11 are highly expressed in the entire cohort. FBXO11 is known to bind and neddylate phosphorylated p53, inhibiting its transcriptional activity while PTPN11 is a tyrosine phosphatase that acts in signal transduction by positively regulating the PI3K/AKT and RAS/MEK/ERK pathways. The next step of this research project is to perform single-cell DNA methylation profiling and single-cell RNA-seq on biopsies from Colombian patients with luminal breast cancer or type I endometrial cancer. This approach will not only help identify new potential biomarkers but also determine which cell types exhibit overexpression of already identified genes. Additionally, it will allow to explore whether the correlation between DNA methylation and gene expression is better at single-cell resolution compared to the bulk resolution used previously.

Single-cell analysis to characterize the potential participation of the NFATC1 gene in cherry angiomas

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Cherry angiomas are skin lesions caused by aggregation of blood vessels. They are typical in aged people but can eventually be confused with amelanotic melanoma. However, molecular differences between these conditions were not fully characterized. In a previous case report, by sequencing the blood, unaffected skin, and angiomas from a patient, a frameshift somatic mutation in the NFATC1 gene was solely found as responsible for these aberrant skin formations.Then we explored single cell skin datasets from the CellXGene database to evaluate the participation of the NFATC1 levels in the blood vessel development. Herein, we observed that endothelial cells are one of the skin cell types with more cells expressing NFATC1 (40%). Using this subset of endothelial cells collected in the skin (14 667 cells), we found that NFATC1-expressing cells show dysregulation of a plethora of genes (around 5 000 with adjusted p-value<0.01 and fold change>2). Subsequently, a gene set enrichment analysis revealed that deregulated genes activate pathways related to the repression of apoptotic processes, regulation of cell adhesion, and blood vessel morphogenesis (adjusted p-value < 0.05). By these results, we accumulate evidence for supporting the participation of mutated versions of NFATC1 in benign tumors previously described in a patient.

Single-Cell RNA-Sequencing for Comprehensive Diagnostics and Therapeutics in Pediatric Ependymoma

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Background: Central Nervous System (CNS) tumors are the leading cause of cancer-related deaths in children, with ependymomas (EPNs) being a significant subtype. These tumors originate from ependymal cells and are classified based on their anatomic location, histology, and molecular profile. Among these, posterior fossa ependymoma (PF-EPN), particularly group A (PF-EPN A), is the most aggressive and affects infants and young children, often with a poor prognosis. Current treatments involve surgery and radiotherapy for children (>3 years), but they carry high risks of recurrence and neuro-cognitive side effects. Moreover, there is a lack of comprehensive genetic information on pediatric brain tumors, particularly regarding specific genetic markers that could aid in early diagnosis and targeted treatment. The urgent need for more targeted and effective treatments is underscored by the intra- and intertumoral heterogeneity and the presence of cancer stem cells (CSCs).
Aims: This propose aims to utilize advanced single-cell RNA sequencing (scRNA-seq) and gene expression technologies to evaluate the total transcriptome of pediatric patients with ependymoma. The goal is to identify predictive markers for diagnosis, prognosis, and precision treatment.
Materials and Methods: Tumor samples from children diagnosed with EPN who underwent surgery at Barretos Cancer Hospital will be selected. These samples will be analyzed using single-cell RNA sequencing (scRNA-seq). Single-cell Gene Expression Flex and Visium HD Spatial Gene Expression will be performed, and sequencing will be conducted on the NovaSeq platform.
Expected Outcome: Single-cell analysis is expected to clarify the organization and interaction of individual cellular components within the tumor. The applications of these advanced methods to classify pediatric CNS tumors will enhance early and accurate diagnosis, benefit clinical management, and contribute to increased survival rates for young patients. Furthermore, this comprehensive understanding may support the development of personalized therapies.

AUSTRAL-Omics: Approaches To Single-Cell Genomics. Study Case: Exploring The Diversity And Dynamics Of Phytoplankton Populations In The Chilean Coast Through Single-Cell Genomics.

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Recent studies have revealed a remarkable diversity of microeukaryotes in diverse ecosystems, many previously undetected, highlighting our lack of knowledge about critical microbial actors in environmental processes such as primary production and nutrient cycling. Single-cell genomics, including scRNAseq, has the potential to obtain genomic informationfrom individual microbes not cultured directly from natural environments. The Chilean coast is subject to changes in environmental variables such as temperature, nutrient levels and pollutants, factors that significantly impact phytoplankton populations, fundamental for the health of marine ecosystems.This study proposes to explore how phytoplankton populations respond to pollution and climate change using single-cell genomics. Phytoplankton, composed of microalgae and cyanobacteria, play a fundamental role in coastal marine ecosystems, acting as the basis of food webs and being responsible for a large part of primary production. However, our understanding of the diversity and dynamics of these microbial communities is still limited. This project seeks to apply single-cell genomics to explore in depth the biodiversity and population structure of phytoplankton in different parts of the Chilean coast. Water samples will be collected at various sites and times of the year, isolating individual phytoplankton cells using flow cytometry. The complete genome of each cell will be amplified and sequenced, allowing the reconstruction of de-novo genomes of non-culturable microorganisms. The genomic data will be analyzed to identify and phylogenetically classify the phytoplankton species present, revealing the existence of previously unknown microbial lineages. Additionally, the abundance and distribution patterns of these populations will be studied along environmental gradients, such as temperature, nutrients and salinity, to understand how they respond to seasonal and long-term fluctuations. This project is feasible thanks to the facilities of AUSTRAL-omics, Core-Facility of UACh, equipped with technology in genomics, transcriptomics and proteomics, including equipment such as NextSeq, MiSeq, Nanopore and MGI.

High-Resolution Profiling of Adipose Tissue Macrophages in Brazilian Populations

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Single-cell transcriptomics enables the capture of multifaceted cell profiles within tissue microenvironments, allowing for the correlation of cell signatures with disease states. We characterized myeloid cells from different human fat depots by profiling them using single-nucleus RNA sequencing (snRNA-seq) and established associations between adipose tissue macrophage (ATM) subpopulations and obesity pathophysiology. Abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue biopsies from seven obese patients (BMI ≥ 35) were collected during bariatric surgery. Nuclei were isolated and subjected to snRNA-seq using the 10x Genomics Single Cell 3' v3 platform. Sequences were processed using Illumina NovaSeq and analyzed with the Cellranger and Seurat platforms. Outliers were removed, and filtering was performed for mitochondrial genes (MT) with a threshold of <20%. The summarized pipeline includes doublet removal (DoubletFinder), ambient RNA removal (DecontX and CellBender), and data integration (scANVI). Six publicly available studies were processed and co-integrated with our datasets to compare myeloid-derived cell populations from our cohort with those reported in the literature. Our study generated one of the largest human ATM atlases, comprising over 15,000 cells clustered into seven subpopulations. We found that patients with a BMI > 25 had higher proportions of a subpopulation we designated Mac4. The Mac1 and Mac3 subpopulations were proportionally correlated with SAT and VAT depots, respectively. Additionally, the Mac7 cluster was more correlated with patients with type 2 diabetes. Gene Set Enrichment Analysis indicated distinct functions for the ATM clusters. Our study contributes to a broader characterization of human ATM heterogeneity and its association with different fat depots and metabolic states. Ethics statement: CAAE:31528820.0.0000.5404

Single-Cell Sequencing To Unravel Microbial Diversity And Metabolic Pathways In Stromatolites From Lake Socompa

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The High Altitude Andean Lakes (HAAL) in the Puna, Central Andes, host microbial communities adapted to extreme conditions like high UV radiation, low oxygen, and high salinity. Modern stromatolites in Lake Socompa (ST), resembling their Precambrian counterparts, were studied using shotgun metagenomics, revealing diverse microbial taxa and metabolic functions across different layers within a 7 mm-deep profile. Upper layers featured aerobic respiration and CO2 fixation pathways dominated by Bacteroidetes, Balneolaeota, Proteobacteria, and Cyanobacteria. Deeper layers showed anaerobic processes, sulfate reduction, and unique metabolic pathways of Firmicutes and Euryarchaeota. Light photoreception and photoprotection profiles varied, with UV-protecting pigments and photoreceptor genes being most prevalent in the upper layers, reflecting adaptations to UV stress and light-driven metabolic activities. Despite these extensive metagenomic studies, traditional culture-based methods limit full community characterization. This project proposes single-cell genome analysis (SCGA) to dissect microbial diversity and metabolic capabilities within stromatolite layers, integrating SCGA data with metagenomics for comprehensive understanding. By sequencing individual cells, we expect to elucidate metabolic pathways, assess horizontal gene transfer impacts, and profile spatial dynamics and symbiotic relationships. In a second phase, integration with transcriptomics will reveal active metabolic processes under varying environmental conditions. This approach promises detailed taxonomic resolution, functional insights, and dynamic microbial interaction networks, advancing our understanding of microbial adaptations to arsenic and UV resistance, and photoreception/phototrophy novel mechanisms. To our knowledge, this is the first application of such an approach to study modern stromatolites in the Puna or elsewhere. This project will advance our understanding of microbial life in extreme environments by expanding our group's methods in omics approaches and bioinformatics capabilities in Tucumán. More important, it will also focus on workforce development in single-cell genomics, fostering collaborations, and enhancing community building in microbial ecology research in Argentina and the region.

Association Between Mutations And Gene Expression Profiles In Malignant Cells Of High-Grade Serous Ovarian Cancer: A Single-Nuclei Rna Sequencing Analysis

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Ovarian cancer is a leading cause of death among gynecological malignancies, with high-grade serous ovarian cancer (HGSOC) being the most prevalent subtype. HGSOC is frequently diagnosed at advanced stages, associated with poor patient's prognosis. Intratumoral heterogeneity, the presence of diverse cancer cell populations within a tumor, is thought to contribute to treatment resistance and disease recurrence. Understanding the genetic alterations driving this heterogeneity is crucial for developing effective therapies. Our study focuses on a cohort of 8 patients diagnosed with HGSOC at the National Cancer Institute (INCA) in Brazil. These patients, aged between 56 and 72 years, were diagnosed between 2014 and 2021 and all presented with advanced-stage disease (III or IV). The patients were divided into two groups: four good responders and four non-responders to chemo. Tumor samples were collected prior to chemotherapy and stored in the National Tumor Bank (BNT-INCA). Single-nuclei RNA sequencing (snRNA-seq) libraries were prepared using the Chromium fixed RNA profiling reagent kits for multiplexed samples (10x Genomics, USA) with the Chromium iX instrument (10x Genomics, USA). Sequencing was performed on the NovaSeq 6000 platform (Illumina, USA). Computational analysis will involve aligning sequencing reads to the reference genome using CellRanger, clustering cells into distinct populations based on gene expression patterns using Seurat, and identifying genetic variants using tools such as SComatic, GATK, and Monopogen. By integrating genetic variant data with gene expression profiles, we will investigate the association between specific mutations and distinct cellular states within malignant subpopulations. Additionally, we will explore the correlation of identified genetic variants with treatment response. This study aims to enhance our understanding of HGSOC biology and assess the feasibility and utility of snRNA-seq as a tool for identifying clinically relevant genetic variants in HGSOC patients.

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