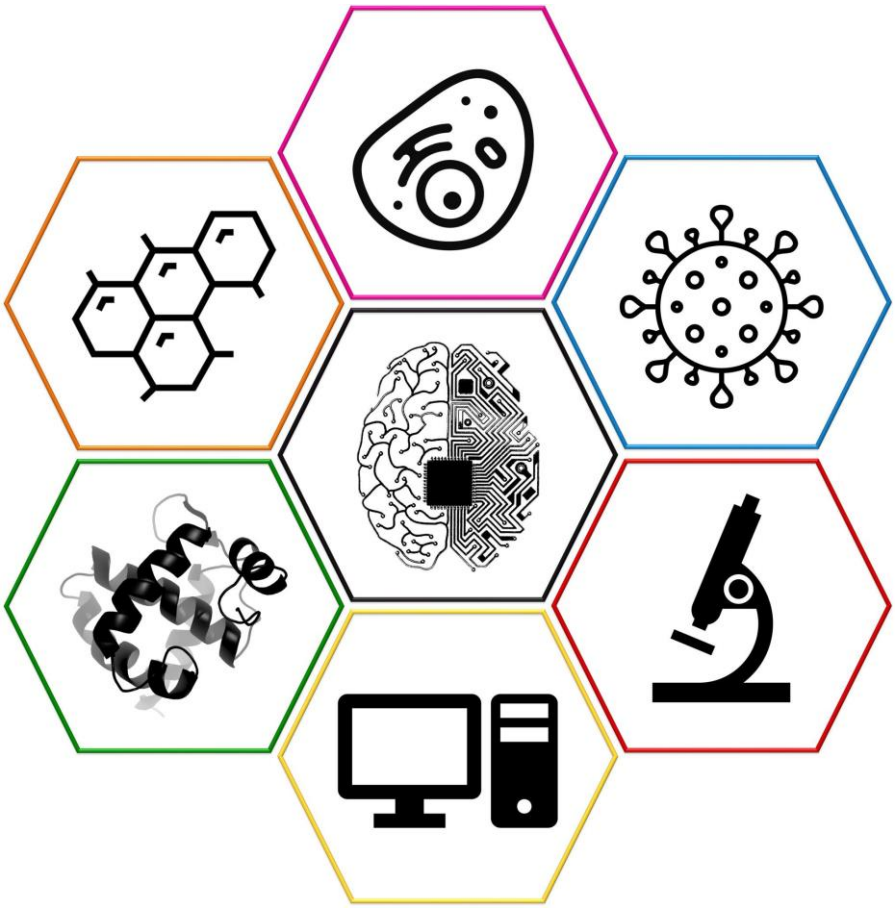
YOUNG MINDS AT WORK: BLENDING BIOLOGY AND BIOINFORMATICS

Abstract Book



On-line conference - December 16th 2022

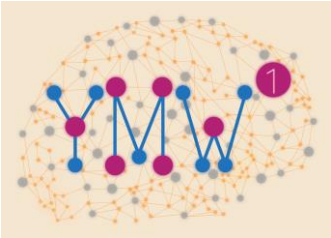


Table of contents

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"Intracranial injection of CIITA-driven MHC-II positive glioblastoma cells induce an effective anti-tumor immune response in vivo: implication for new therapeutic strategies against the glioblastoma"

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"Glicopro, a multi-modulative ocular formulation based on standardized and sterile snail mucus extract"

Poster

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"Identification of trans factors regulating Tau exon 6 alternative splicing"

Maria Vittoria Morone

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"HPV16: first interactive map between human proteins and the viral genome"

Abstracts

Oral Microbiome Dysbiosis Is Associated With Symptoms Severity and Local Immune/Inflammatory Response in COVID-19 Patients: A Cross-Sectional Study

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The human oral microbiome (HOM) is the second largest microbial community after the gut and can impact the onset and progression of several localized and systemic diseases, including those of viral origin, especially for viruses entering the body via the oropharynx [1-3] including the pandemic human coronavirus SARS-CoV-2, which causes the COVID-19 disease [4]. The aim of the present study was therefore to characterize the HOM in COVID-19 patients, including its non-bacterial components (mycome, virome), to evidence the association between virus-induced disease and the microbial environment of the oral cavity. Moreover, the inflammation and local immune response were also assessed in parallel.

Seventy-five oral rinse samples were analyzed by Whole Genome Sequencing (WGS) to simultaneously identify oral bacteria, fungi, and viruses. To correlate the HOM profile with local virus replication, the SARS-CoV-2 amount in the oral cavity was quantified by digital droplet PCR. In parallel, the local immune response (secretory IgA) and inflammatory cytokine release (IL-6, IL-17, TNFα, and GM-CSF) were assessed by specific ELISA assays.

The results showed the presence of oral dysbiosis in COVID-19 patients compared to matched controls, with significantly decreased alpha-diversity value and lower species richness in COVID-19 subjects. Bacterial genera associated with poor oral hygiene and periodontitis were increased in COVID-19 group (Prevotella, Capnocytophaga, Porphyromonas, Abiotrophia, Aggregatibacter), and Enterococcus and Enterobacter spp. were exclusively detectable in COVID-19 patients. Mycetes (Candida, Saccharomyces) and viruses (EBV, HSV-1) were also significantly increased, with Aspergillus, Nakaseomyces, and Malassezia genera exclusively detectable in COVID-19 patients. Notably, oral dysbiosis correlated with symptom severity ($p = 0.006$) and increased local inflammation ($p < 0.01$). In parallel, a decreased mucosal sIgA response was observed in more severely symptomatic patients ($p = 0.02$).

The oral microbiome may be important in defining the individual susceptibility to SARS-CoV-2 infection and the subsequent development of symptomatic COVID-19. In particular, poor oral hygiene might facilitate inflammation and a worse course of COVID-19 disease. Instead, sIgA presence associated with mild symptoms may be considered as an important marker in monitoring therapy and vaccine development.

References

1. Wade, W. G. (2013). The oral microbiome in health and disease. Pharmacol. Res. 69,137–143. doi: 10.1016/j.phrs.2012.11.006
2. Baghbani, T., Nikzad, H., Azadbakht, J., Izadpanah, F., and Haddad Kashani, H. (2020). Dual and mutual interaction between microbiota and viral infections: a possible treat for COVID-19. Microb. Cell Fact. 19:217. doi: 10.1186/s12934-020-01483-1
3. Caselli, E., Fabbri, C., D'Accolti, M., Soffritti, I., Bassi, C., Mazzacane, S., et al. (2020). Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol. 20:120. doi: 10.1186/s12866-020-01801-y
4. Iebba V, Zanotta N, Campisciano G, Zerbato V, Di Bella S, Cason C, Luzzati R, Confalonieri M, Palamara AT, Comar M. Profiling of Oral Microbiota and Cytokines in COVID-19 Patients. Front Microbiol. 2021 Jul 30;12:671813. doi: 10.3389/fmicb.2021.671813

Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency

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The COVID-19 pandemics has highlighted how much the public transportation environment, such as subways, may be important for transmission of potential pathogens. Consistent with this, based on massive use of chemical disinfection has been mandatorily introduced during the SARS-CoV-2 emergency, and is still in place [1]. However, chemical disinfectants have a temporary action and could worsen both pollution and antimicrobial resistance (AMR) concerns. Alternatively, a probiotic-based sanitation (PBS) was recently shown to provide effective and long-term pathogen control (including SARS-CoV-2) without worsening AMR spread [2,3]. Thus, our study was aimed to assess the PBS effect on the subway microbiome, compared to chemical disinfectants, during the COVID-19 pandemics.

Two underground trains were enrolled in the study: one train (control) continued to receive conventional chemical sanitation, whereas the other train received PBS in substitution of chemical disinfection. The trains' microbiome was characterized by both culture-based (CFU count) and molecular methods, including 16S rRNA NGS for bacteriome characterization, qPCR microarray for resistome characterization, and digital droplet PCR for
SARS-CoV-2 monitoring.

The results showed a stable >80% reduction of bacterial/fungal pathogens ($P<0.001$), of AMR ($Pc<0.01$) and of SARS-CoV-2 presence ($P<0.01$) in the PBS-treated train compared with the chemically disinfected control train. NGS bacteriome profiling evidenced diverse clusters in the air vs. surface microbiomes, meanwhile demonstrating the specific action of PBS against pathogens rather than the entire train bacteriome.

The data presented provide the first direct assessment of the impact of different sanitation procedures on the subway microbiome, allowing a better understanding of its composition and dynamics and showing that a biological sanitation approach may be highly effective in counteracting pathogens and AMR spread in our increasingly urbanized and interconnected environment.

References

1. CDC 2021, CDC. Cleaning and Disinfecting Your Facility. 2021. https://www.cdc.gov/coronavirus/2019-ncov/community/disinfecting-building-facility.html.
2. Caselli E, Arnoldo L, Rognoni C, D'Accolti M, Soffritti I, Lanzoni L, Bisi M, Volta A, Tarricone R, Brusaferro S, Mazzacane S. Impact of a probiotic-based hospital sanitation on antimicrobial resistance and HAI-associated antimicrobial consumption and costs: a multicenter study. Infect DrugResist. 2019; 12:501-510;
3. D'Accolti M, Soffritti I, Bonfante F, Ricciardi W, Mazzacane S, Caselli E. Potential of an Eco-Sustainable Probiotic-Cleaning Formulation in Reducing Infectivity of Enveloped Viruses.Viruses.2021;13:11.

NGS analysis of nasopharyngeal microbiota in SARS-CoV-2 positive patients during the first year of the pandemic in the Campania Region of Italy

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Since its first appearance, SARS-CoV-2 rapidly spread all over the world, reaching the pandemic scale and causing, to date, more than 630 million confirmed infections and 6 million of deaths. One of the major threats in Covid-19 patients is represented by bacterial secondary infections that lead to a worsening of the clinic picture with high risk of death. Thus, there is an urgent need of understanding the correlation between SARS-CoV-2 effects and bacterial superinfections, to take a prompt action. The principal aim of our study is to investigate existing correlations in the nasopharynx between the bacterial community, potential pathogens, and SARS-CoV-2 infection. The main aim of this study was to provide evidence pointing to possible relationships between components of the bacterial community and SARS-CoV-2 in the nasopharynx.

Meta-transcriptomic profiling of the nasopharyngeal microbial community was carried out by RNA-seq in 89 SARS-Cov-2 positive subjects from the Campania Region in Italy during 3 different seasonal periods of COVID-19 pandemic. The 89 patients were divided in different groups based on the season, age, gender, and symptomatology. These parameters were matched with the presence of bacterial superinfections and correlated with the severity of the illness.

Results show a consistently high presence of members of the Proteobacteria (41.85%), Firmicutes (28.54%), and Actinobacteria (16.10%) phyla, and an inverted correlation between the host microbe, co-infectious bacteria, and super-potential pathogens such as Staphylococcus aureus (98%), Klebsiella pneumoniae (80%), Streptococcus pneumoniae(52%), Pseudomonas aeruginosa (19%), Acinetobacter baumannii (16%), and Neisseria gonorrhoeae (79%).

In conclusion this study shows that, although the balance between the microbes that populate the nasopharynx is very complex, as subject to variables , there is significant relationship between the composition of the microbiota of SARS-Cov-2 positive and negative control patients and a clear correlation between high SARS-cov-2 load and proliferation of super-pathogenic bacterial species such as Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii, accompanied by a reduced abundance and bacterial diversity in the nasopharynx.

Short peptides inhibit SARS-CoV-2 infection

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Recently, the research has been animated by the pressing need for therapeutic and prophylactic treatments against the infection caused by SARS-CoV-2. Peptide inhibitors are a valid alternative approach for treating emerging viral infections mainly due to their low toxicity, easy synthesis and high efficiency. The present study was focused on two short peptides, each consisting of only three amino acid residues, deriving from two recurrent nucleotide signatures recently identified in many pathogenic microorganisms [1].

Several viral genomes were analyzed in order to search the two recurrent nucleotide sequences. The amino acids produced were synthesized and, the stability and cytotoxicity of peptides were evaluated. In addition, antiviral activity and molecular docking were performed.

Surprisingly, the peptides inhibited the infection of both the human coronavirus OC43 and SARS-CoV-2. Their small size did not allow their structuring, but we observed that both could bind the RBD of the spike protein, as suggested by molecular docking and validated by biochemical studies. Our results demonstrated that tripeptides could occupy pockets inside S1 and S2 domains, potentially blocking all downstream events and the entire SARS-CoV-2 replication cycle.

The two tripeptides inhibit beta coronaviruses entry mechanism probably by interfering with the viral spike protein. In addition, peptides had a very low toxicity profile and a long half-life in serum, suggesting their potential use as innovative and safe options in the prevention and antiviral therapy against SARS-CoV-2 infection.

References

1. D'Angelo G. and Palmieri F. Discovering genomic patterns in SARS-CoV-2 variants. Int J Intell Syst 35(11):1680–1698.

Decay of neutralization activity in vaccinees receiving anti-SARS-CoV-2 3rd booster dose BNT162b2

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Anti-SARS-CoV-2 vaccination uses the Spike (S) viral protein to induce the production of neutralizing antibodies (NAbs) that block the Receptor Binding Domain (RBD) preventing virus entry into the host cell [1]. NAbs are known to fade over time after vaccination naturally. Besides, studies are still ongoing to evaluate the extent of this decrease with SARS-CoV-2 vaccine [2]. We studied neutralizing activity against Wuhan and emerging variants of concerns (VoC) BA.1, BA.2, BA.2.75, BA.4/5, before and after the 3rd booster dose with BTN162b2.

Our study group included 17 Health Care Workers (HCW) who received the 3rd vaccine dose(BNT162b2): 14 were naïve to infection (Naïve); 3 had evidence of previous infection (PI). We collected serum samples before, one month and 4 months after the booster dose administration. We evaluated anti-S IgGs using enzyme-linked immunosorbent assay (ELISA) and neutralizing activity using pseudovirus neutralisation assays (PVNA). SARS-CoV-2 PVs carrying Luciferase reporter gene were generated in HEK293T cells, harvested after 72hs, titred and stored at -80 before use. For the PVNA: viruses were incubated with serum for 1h, at 37°C, 5% CO2, before incubation with ACE-2/TMPRSS2-expressing CHO target cells for 48hs and read out of Luciferase signal [3]. Neutralizing potential was expressed as inhibitory concentration 50 (IC50).

Overall neutralizing activity against all viruses significantly increases within the first month after vaccine administration but declines after 16 weeks. Anti-Wuhan neutralizing activity was the most efficient, the BA.1 variant was the least neutralized, at each time point. Overall, PI vaccinees showed higher neutralization activity against all viruses up to 4 months of follow-up compared to Naïve ones. No effects in the neutralizing activity were observed related to sex or age. Anti-S IgG titre rises proportionally to the increment of the IC50 against Wuhan, BA.1 and BA.2.75 variants.

Our preliminary results on a cohort of 17 HCW showed that 3rd booster dose induced a quick rise in neutralization activity, that decreased over time, with the average IC50 nearly halved at 16 weeks follow-up. Previous exposure to the virus gives a slightly mild long-term
neutralization advantage. IC50 and anti-S IgG titre are directly correlated.

References

1. Carrillo, J. et al. Humoral immune responses and neutralizing antibodies against SARS-CoV-2; implications in pathogenesis and protective immunity. Biochem. Biophys. Res.Commun. 538, 187–191 (2021).
2. Trombetta, C. M. et al. Immune response to SARS-CoV-2 Omicron variant in patients and vaccinees following homologous and heterologous vaccinations. Commun. Biol. 2022 51 5,1–7 (2022).
3. Di Genova, C. et al. Production, titration, neutralisation, storage and lyophilisation of severe acute respiratory syndrome coronavirus 2 (sars-cov-2) lentiviral pseudotypes. Bio-protocol 11, (2021).

An integrated multidisciplinary platform to develop and validate anti-COVID-19 compounds

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The unexpected spread of the COVID-19 pandemic highlighted the crucial need for finer preparedness and responsiveness to epidemic-prone pathogens. The lack of global coordination and insufficient application of integrative approaches must be addressed in the shortest time. This is not a trivial intention since the computational approaches applied to rapidly screen many molecules provided, in most cases, controversial data. We developed and validated an integrated multidisciplinary platform for the design and testing of the in vitro effect of anti-Covid-19 compounds selected from in silico studies.

The platform involved the design and synthesis of novel peptides/peptidomimetics able to bind to the Receptor Binding Domain (RBD), full-length SARS-CoV-2 Spike protein (S), its S1/S2 subdomains and/or human Angiotensin-Converting Enzyme-2 (ACE2). Rosetta Peptiderive software was initially used to identify and select the inhibitor peptides at Spike-RBD/ACE2 interface. The ability of the new peptides to bind to the relevant domains was tested by using Surface Plasmon Resonance and ELISA approaches. The binding affinity of natural compounds (i.e., limonene, menthol, magnolol, and licorice derivatives) as well as drugs already used in clinics belonging to different classes (i.e., anti-inflammatory, antipsychotic, and antibiotic) was also investigated. The most promising candidates were then assessed in cell transduction assays in the presence of lentiviruses expressing S full-length or the more widespread variants.

Based on the RBD sequence and the predictive structure at the interface between RBD and ACE2, we designed, synthesized, and optimized more than 40 new peptides being tested for their ability to interfere with S-ACE2 binding. Among them, we identified a cyclic peptide able to reduce infectivity in vitro. Moreover, two other different molecules (an antipsychotic and an estrogen receptor modulator) showed a strong capability to counteract the cellular transduction of pseudotyped lentivirus exposing all the SARS-CoV-2 Spike isoforms tested.

The developed integrated multidisciplinary platform was effective in designing and testing the anti-COVID-19 activity of compounds and allowed the identification of a promising new peptide and two already approved drugs.

References

- Diomede et al. 2021. Viruses

Role of CD147 in promoting SARS-CoV-2 in situ infection and anticoagulant PROS1 alteration

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Coagulation activation accompanied by excessive immune and inflammatory reactions and thrombosis, represents the second leading cause of death following SARS CoV-2 infection. The ability of SARS-CoV-2 to infect endothelial cells, thanks to the expression of ACE2 and CD147 receptors on their surface, cause an important imbalance of hemostatic system. One of the main anticoagulant factors altered during COVID-19 disease is the protein S (PROS1), known to be expressed by endothelium. Thus, SARS-CoV-2 infection of endothelial cells could directly damage the endothelium and alter the production of PROS1 via proteolytic cleavage by viral PL-pro, leading to coagulopathies [1-6].

The study was conducted on coagulopathic patients, 7 with previous or ongoing SARS-CoV-2 infection, documented as positive to Rel-Time PCR swab test, that experienced a clinical COVID-19, and 11 controls. The expression of SARS-CoV-2 Nucleoprotein (NP), ACE2 and CD147 was evaluated in thrombi and venous/arterial tissue samples by immunohistochemistry and SARS-CoV-2 RNA, ACE2 and CD147 mRNA was detected by Real-Time PCR. The levels of PROS1 were analyzed in plasma samples using ELISA assay.

SARS-CoV-2 genome was identified in both thrombi and venous/arterial tissue samples of COVID-19 patients, with the highest viral load at thrombotic level. The presence of SARS-CoV-2 in the samples analyzed correlates with a previous infection at the time of sampling. ACE2 and CD147 mRNA expression was higher in COVID-19 patients compared to controls ($p<0.001$). The analysis of proteins expression by IHC confirmed a higher expression of CD147 in COVID-19 patients in comparison with controls, particularly in thrombi that presented a positive staining for SARS-CoV-2 NP ($p<0.01$). PROS1 levels were lower in COVID-19 subjects in comparison to controls ($p=0.015$).

The presence of SARS-CoV-2 NP and RNA expression in both thrombi and venous/arterial tissue samples of COVID-19 patients suggest a possible role in coagulopathies onset, as confirmed by the decrease in anti-coagulant PROS1 levels in COVID-19 patients. The entry pathway seems to engage CD147, that co-localizes with tissue SARS-CoV-2 NP positivity, especially at thrombotic level. These data support further researches to identify possible therapeutic targets that can control the involvement of SARS-CoV-2 infection in coagulopathies.

References

- Iba, T., et al., Coagulopathy of Coronavirus Disease 2019. Crit Care Med, 2020. 48(9): p. 1358-1364.
- Jose, R.J. and A. Manuel, COVID-19 cytokine storm: the interplay between inflammation and coagulation. The Lancet Respiratory Medicine, 2020. 8(6): p. e46-e47.
- Bortolotti, D., et al., Relevance of VEGF and CD147 in different SARS-CoV-2 positive digestive tracts characterized by thrombotic damage. FASEB J, 2021. 35(12): p. e21969.
- Ganier, L., X. Morelli, and J.P. Borg, CD147 (BSG) but not ACE2 expression is detectable in vascular endothelial cells within single cell RNA sequencing datasets derived from multiple tissues in healthy individuals. Med Sci (Paris), 2020. 36 Hors serie n degrees 1: p. 42-46.
- Tutusaus, A., et al., Role of Vitamin K-Dependent Factors Protein S and GAS6 and TAM Receptors in SARS-CoV-2 Infection and COVID-19-Associated Immunothrombosis. Cells, 2020. 9(10).
- Castoldi, E. and T.M. Hackeng, Regulation of coagulation by protein S. Curr Opin Hematol, 2008. 15(5): p. 529-36.

Efficacy of COVID-19 mRNA vaccination in patients with autoimmune disorders: humoral and cellular immune response

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We analyzed humoral and T cell-mediated response after COVID-19 mRNA vaccine in immunosuppressed patients and patients with common variable immunodeficiency disease (CVID). We enrolled 38 patients and 11 healthy sex- and age- matched controls. Four patients were affected by CVID and 34 by chronic rheumatic diseases (RDs).

All patients with RDs were treated by corticosteroid therapy and/or immunosuppressive treatment and/or biological drugs: 14 patients were treated with abatacept, 10 with rituximab and 10 with tocilizumab. Total antibody titer to SARS-CoV-2 spike protein was assessed by electrochemiluminescence immunoassay, CD4+ and CD4+-CD8+ T cell-mediated immune response was analyzed by interferon-γ release assay, the production of IFN-γ-inducible and innate-immunity chemokines by cytometric bead array after stimulation with different spike peptides. The expression of CD40L, CD137, IL-2, IFN-γ and IL-17 on CD4+ and CD8+ T cells, evaluating their activation status, after SARS-CoV-2 spike peptides stimulation, was analyzed by intracellular flow cytometry staining.

The main findings were a reduced anti-S response in ABA-treated group, restored after the third dose of vaccine; an impaired T cell activation, represented by a reduction of IFN-γ and related chemokines; a reduction of effector memory CD8 T cells in ABA-treated group; a significant ability of ABA treated group to mount a CD4 T cell response, when stimulated with spike derived antigens.
Our work was limited by the low number of patients enrolled but performing extended cellular assessments, contributed to explain which kind of immune response patients chronically exposed to different immunosuppressive regimen are able to generate in response to COVID-19 vaccination. The preserved ability to generate clones of CD4+ T lymphocytes specific for SARS-CoV-2 Spik proteins represents the assurance of an effective protection of vaccination to SARS-CoV-2. Moreover, after the third dose of COVID-19 mRNA vaccine, ABA-treated patients acquired the capability to produce a strong antibody response, despite they maintained a significant reduction of CD8+ T response.

All these data represent a critical message from laboratory research bench to clinical patients' side, suggesting that repeated vaccine doses may be necessary to optimize the immunological response and to induce stronger serological responses in these high-risk vulnerable patients.

References
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Dynamics of nasopharyngeal tract phageome and association with disease severity and age of patients during three waves of COVID-19

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The COVID-19 pandemic is a global outbreak of coronavirus, an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several evidence report the crucial role of the microbiota in disease severity, age groups of patients and pandemic temporal evolution1. One of the most underestimated aspects of the microbiome is bacteriophages. The current study investigated the phageome profiles under the three mentioned conditions.

Fifty-five SARS-CoV-2 positive patients from the Campania region were included in this study. Nasopharyngeal swab samples were collected during the three main SARS CoV-2 outbreaks in Italy: March-May 2020 (n.25); September-November 2020 (n.25); January-February 2021 (n.5). Furthermore, they were divided according to the severity of symptoms into non-serious (n.39), moderate (n.6) and severe (n.10)2,3. Enrolled patients ranged in age from 8 to 91 years. RNA was extracted using ELITeInGenius system. RNA samples were sequenced on the NextSeq 500 and phage abundances were analyzed via HOME-BIO 15 software.

A total of 6 phage families were detected in 55 nasopharyngeal swabs taken from COVID-19 patients. Siphoviridae was the most frequently encountered family, followed by Myoviridae. Focusing on the three different pandemic waves, Peduovirinae was more abundant in wave I than in wave II. The same trend was found for the Autographiviridae and Microviridae families. Similarly, Peduovirinae, Autographiviridae and Microviridae were more frequent in the first period than in the third. The different phage families were more detected in the samples of patients with severe symptoms than in those classified as non severe. Siphoviridae, Myoviridae, and Microviridae were less abundant in the symptom-free patients than in the severe group. When compared across different age groups, only 2 phage families showed significant differences. Specifically, Autographiviridae and Siphoviridae were less abundant in patients aged 41 to 59 years than in all other groups.

During SARS-CoV-2 infection, there are significant changes in phagoma composition in the nasopharyngeal tract. During SARS-CoV-2 infection, there are significant changes in phagoma composition in the nasopharyngeal tract. This evidence could be used to formulate better management strategies in the fight against COVID-19. 2.

References

- De R., Dutta S. Role of the Microbiome in the Pathogenesis of COVID-19. Front Cell Infect Microbiol. 2022; 12: 736397.
- Giugliano R., Sellitto A., Ferravante C., Rocco T., D'Agostino Y., Alexandrova E., Lamberti J., Palumbo D., Galdiero M., Vaccaro M., Pagliano P., Weisz A., Giurato G., Franci G., Rizzo F. NGS analysis of nasopharyngeal microbiota in SARS-CoV-2 positive patients during the first year of the pandemic in the Campania Region of Italy. Microb Pathog. 2022, 165:105506.
- Ferravante C. Sanna G., Melone V., Rocco T., D'Agostino Y., Lamberti J., Alexandrova E., Pecoraro G., Pagliano P., Astorri R., Manzin A., Weisz A., Giurato G., Galdiero M., Rizzo F., Franci G. Nasopharyngeal virome analysis of COVID-19 patients during three different waves in Campania region of Italy. J Med Virol 2022, 94(5):2275-2283.

Strong Humoral Immune Response induced by Heterologous Prime–Boost Vaccination with ChAdOx1 nCoV-19 and BNT162b2 in a Sardinian cohort group: preliminary data

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The emergence and rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant, able to escape vaccine-induced immunity pose a serious concern on the vaccine coverage and the impact within populations (1).

Quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG) and neutralization assay (NA) were run to investigate, in the long-term period (4 and 20 weeks after the second dose and 4 weeks after the booster dose), the responses to vaccine treatment in a subgroup of our cohort of Sardinian subjects with heterologous priming with ChAdOx1 (ChAd) vector vaccine, followed by boosting with BNT162b2 (ChAd/BNT/BNT) and ChAd/ChAd/BNT).
We also analyzed the neutralization titers against the SARS-CoV-2 wt and Omicron BA.1 variant of a subgroup of participants that tested positive for SARS-CoV-2, after the completion of the vaccination regimen.

Heterologous regimen provided a more robust antibody response than either of the homologous regimens. After the booster dose homologous and heterologous vaccination provided a strong and comparable antibody response. From our data, after natural infection, in the BNT-BNT-BNT group, the neutralization titers showed against the BA.1 variant, a notable decrease when compared to that determined against wt SARS-COV-2 strain. The neutralization titers in the ChAd-BNT-BNT and ChAd-ChAd-BNT group, against wt and BA.1 strains resulted in comparable neutralization activity and no reduction were detected against the Omicron BA.1 strain.

These data may be of interest and provide additional support in case of concerns among vaccine supply, but further assessments after the 4th dose are needed on the magnitude of the immune response, crucial for updating SARS-CoV-2 vaccine strategies.

References

- Markus Hoffmann, Nadine Krüger, Sebastian Schulz, Anne Cossmann, Cheila Rocha, Amy Kempf, Inga Nehlmeier, Luise Graichen, Anna-Sophie Moldenhauer, Martin S. Winkler, Martin Lier, Alexandra Dopfer-Jablonka, Hans-Martin Jäck, Georg M.N. Behrens, Stefan Pöhlmann, The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic, Cell. 2022 Feb 3;185(3):447-456.e11

Introduction of probiotic-based sanitation in the emergency ward of a children's hospital during the covid-19 pandemic

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Antimicrobial resistance (AMR) represents a major threat to public health, especially in the hospital environment, and the massive use of disinfectants to prevent COVID-19 transmission have intensified this concern. However, the control of microbial
contamination is crucial in hospitals, since the hospital microbiome is a reservoir of pathogens responsible for healthcare-associated infections (HAIs), which are particularly frequent and severe in pediatric wards [1]. Since we had previously reported that a probiotic-based sanitation (PBS) could stably decrease pathogens, their AMR, and HAIs in hospitals for adults [2,3], this study aimed to characterize the microbial contamination in the emergency rooms (ER) of a children's hospital during the COVID-19 pandemic, and to assess the effect of PBS sanitation compared to chemical disinfection.

For two months, PBS replaced the conventional chemical disinfection in routine sanitation. Microbial contamination was monitored before PBS introduction (T0) and at 2, 4, and 9 weeks after PBS introduction. by both culture-based (CFU count) and molecular methods, including 16S rRNA NGS for bacteriome characterization and real time PCR (qPCR) microarrays for the evaluation of the resistome of the contaminating population. The presence of SARS-CoV-2 RNA genome was also monitored by specific qRT-PCR.

As expected, the results showed substantial pathogen contamination and presence of several AMR genes in the ER environment at T0. Afterwards, PBS usage induced a stable 80% decrease of surface pathogens compared to the T0 levels ($P < 0.01$), and an up to 2 log decrease of AMR genes ($Pc < 0.01$). The effects were reversed when reintroducing chemical disinfection. SARS-CoV-2 was never detectable. NGS results showed that PBS induced a bacteriome remodulation, with decreased relative abundance of Staphylococcus spp. ($P < 0.001$). Streptococcus spp. ($P < 0.001$), Escherichia/Shigella spp. ($P < 0.01$), Acinetobacter spp. and Pseudomonas spp. (although not statistically significant).

The control of hospital biodurden is a major issue, and collected data highlight the potential of molecular methods in describing precisely the type of microbial contamination and suggest that PBS may be successfully used to limit both pathogen and AMR spread.

References

- Lake JG, Weiner LM, Milstone AM, Saiman L, Magill SS, See I. Pathogen distribution and antimicrobial resistance among pediatric healthcare-associated infections reported to the national healthcare safety network, 2011–2014. Infect Cont Hosp Epidemiol. 2018;39(1):1– 11. doi:10.1017/ice.2017.236
- Caselli E, D'Accolti M, Vandini A, et al. Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One. 2016;11(2):e0148857. doi:10.1371/journal.pone.0148857
- aselli E, Brusaferro S, Coccagna M, et al. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: a multicentre, prospective, intervention study. PLoS One. 2018;13(7):e0199616. doi:10.1371/journal.pone.0199616

SARS-CoV-2 omicron variant intra-host quasispecies in acute infection: a novel approach for contact tracing

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The continuous spread of SARS-CoV-2 across the world has allowed the virus to generate variants with various levels of transmissibility and severity. Whole genome sequencing has been critical to map outbreaks. For an easier analysis, a consensus sequence has been considered for each infected individual. Nevertheless, it is important to consider that RNA virus transmitted is not represented by a single sequence, but consists of a heterogeneous population designated as quasi-species, whose transmission was governed by a bottle-neck.1 High-throughput sequencing of the intra-host viral populations during a chronic infection supported by RNA viruses, such as HCV, has become a tool for outbreak investigations.2In the SARS-CoV-2 omicron era, a novel approach based on intra-host single nucleotide variants (iSNVs) analysis could be useful to support contact tracing.

A serological follow-up was conducted on 85 healthcare workers employed in the Microbiology unit of the Brescia Civic Hospital. Timelines of SARS-CoV-2 positive cases were constructed to generate hypotheses on the spread of infection. Nasopharyngeal swabs were collected from 18 subjects, undergoing symptomatic infection despite completion of the vaccination cycle. Total RNA was sequenced using the Paragon Genomics' CleanPlex multiplex PCR Panel. The deep sequencing was performed on MiSeq platform and raw data were analyzed with the SOPHiA GENETICS' SARS CoV-2 Panel. The variant calling was carried out by the Variant Finder Tool (Geneious). Biological effects were annotated using snpeff.

Using iSNVs, we were able to follow the transmission during SARS-CoV-2 acute infections in immunocompetent, fully vaccinated, individuals. Genetic screening revealed 1122 iSNVs, mainly scattered over ORF1ab and S genes. We established the donor and the recipient individuals using the date of symptom onset and inferred transmission bottleneck sizes using the beta-binomial inference method.3 Our analysis, in 6 well-established pairs, estimated mean bottleneck sizes ranging from 37 to 200. The cluster analysis performed with iSNVs found along the genome correctly identified epidemiologically linked individuals. We further evaluated that the iSNVs found in the NSP2, ORF3 and ORF7 genes are sufficient to difference the transmission dynamics.

In our opinion, these three genes, due to their genetic stability, are the perfect candidates in contact tracing analysis.

References:

- Caccuri F, Messali S, Bortolotti D, Di Silvestre D, De Palma A, Cattaneo C, Bertelli A, Zani A, Milanesi M, Giovanetti M, Campisi G, Gentili V, Bugatti A, Filippini F, Scaltriti E, Pongolini S, Tucci A, Fiorentini S, d'Ursi P, Ciccozzi M, Mauri P, Rizzo R, Caruso A. Competition for dominance within replicating quasispecies during prolonged SARS-CoV-2 infection in an immunocompromised host. Virus Evol. 2022 May 21;8(1):veac042. doi: 10.1093/ve/veac042. PMID: 35706980; PMCID: PMC9129230.
- Skums P, Zelikovsky A, Singh R, Gussler W, Dimitrova Z, Knyazev S, Mandric I, Ramachandran S, Campo D, Jha D, Bunimovich L, Costenbader E, Sexton C, O'Connor S, Xia GL, Khudyakov Y. QUENTIN: reconstruction of disease transmissions from viral quasispecies genomic data. Bioinformatics. 2018 Jan 1;34(1):163-170. doi: 10.1093/bioinformatics/btx402. PMID: 29304222; PMCID: PMC6355096.
- Braun KM, Moreno GK, Wagner C, Accola MA, Rehrauer WM, Baker DA, Koelle K, O'Connor DH, Bedford T, Friedrich TC, Moncla LH. Acute SARS-CoV-2 infections harbor limited within-host diversity and transmit via tight transmission bottlenecks. PLoS Pathog. 2021 Aug 23;17(8):e1009849. doi: 10.1371/journal.ppat.1009849. PMID: 34424945; PMCID: PMC8412271.

Evaluation of KIR2DL2 and HLA-C1 alleles frequency in COVID-19 patients

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Severe COVID-19 patients are characterized by anergic Natural Killer (NK) cells (1) and a consequent lower ability to control of SARS-CoV-2 infection. NK cells play a crucial role in the control of viral infection and their activity is regulated by several inhibitory receptors, that might be exploited by viruses as an immune-escape mechanism. In particular, the
inhibitory Killer Immunoglobulin-Like Receptors (KIRs) KIR2DL2 and its ligand Human Leucocyte Antigen- C (HLA-C) (2,3) have been already described as associated to increased susceptibility to viral infections (4,5). Due to the central role of NK cells in the antiviral response and their anergic status described in COVID-19 patients, the aim of this study was to investigate the possible involvement of KIR2DL2 inhibitory receptor and its ligand HLA-C1 in the susceptibility to SARS-CoV-2 infection and in COVID-19 outcome.

Blood samples from 109 COVID-19 subjects, determined by Real-Time PCR swab detection, and from 110 healthy controls have been collected and DNA extracted for KIR2DL2 and HLA-C1 genotyping by PCR. The results were correlated with clinical and demographical data.

COVID-19 subjects showed a higher allele frequency compared to controls of both KIR2DL2 (64,2% vs 36,4 %, respectively, $p<0.0001$) and HLA-C1 (81% vs 52%, respectively, $p<0.001$). KIR2DL2 frequency was found increased in COVID-19 positive women compared to men (72% vs 57% in men, $p<0.05$). Considering the age of COVID-19 population, the results showed a positive correlation between age and KIR2DL2 frequency ($p<0.05$), with the highest frequency in elder subjects. Furthermore, we observed an increased frequency of both KIR2DL2 and HLA-C1 alleles in COVID-19 subjects who worsen and consequently died (61%, $p<0.05$) compared to control subjects, with a higher frequency of HLA-C1 allele in the subjects that needed Intensive Care Unit (ICU) (73%, $p<0.0001$).

The present study suggests that KIR2DL2 and HLA-C1 alleles are highly represented in COVID-19 patients, with an increased frequency in patients with a severe disease. These results suggest a possible increase in KIR2DL2 and HLA-C1 alleles expression, that might lead to NK cell anergy and consequent increase of SARS-CoV-2 susceptibility and disease severity, evident mainly in women and elderly subjects.

2. References

- Ghasemzadeh M., Ghasemzadeh A., Hosseini E., Exhausted NK cells and cytokine storms in COVID-19: Whether NK cell therapy could be a therapeutic choice. Human Immunology 2021, 83(1): 86–98. https://doi.org/10.1016/j.humimm.2021.09.004.
- van Erp, E.A.; van Kampen, M.R.; van Kasteren, P.B.; de Wit, J. Viral Infection of Human Natural Killer Cells. Viruses 2019, 11, 243. https://doi.org/10.3390/v11030243. 3. Uhrberg M., Valiante N.M., Shum B. P., Shilling H. G., Lienert-Weidenbach K., Corliss B., Dolly T., Lanier L, Parham P., Human Diversity in Killer Cell Inhibitory Receptor Genes. Immunity 1997, https://doi.org/10.1016/S1074-7613(00)80394-5.
- R. Rizzo et al., KIR2DS2/KIR2DL2/HLA-C1 Haplotype Is Associated with Alzheimer's Disease: Implication for the Role of Herpesvirus Infections, J. Alzheimer's Dis., vol. 67, no. 4, 2019, doi: 10.3233/JAD-180777.
- Bortolotti, D.; Gentili, V.; Bortoluzzi, A.; Govoni, M.; Schiuma, G.; Beltrami, S.; Rizzo, S.; Baldi, E.; Caselli, E.; Pugliatti, M.; Castellazzi, M.; Fernández, M.; Fainardi, E.; Rizzo, R. Herpesvirus Infections in KIR2DL2-Positive Multiple Sclerosis Patients: Mechanisms Triggering Autoimmunity. Microorganisms 2022, 10, 494. https://doi.org/10.3390/microorganisms10030494.

Tissue positivity for SARS-CoV-2 N-Protein in a preterm born infant death of thrombosis: a possible case of intrauterine transmission

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COVID-19 (COronaVIrus Disease 2019) systemic clinical manifestations might include inflammation and pro-coagulative processes, leading to thromboembolic events (TE) generally observed in severe COVID-19 patients and recently discovered in children [1]. Moreover, although the rate of mother-to-newborn transmission is less than 5% [2], some cases of SARS-CoV-2 positive newborns with early-onset symptoms were reported [3], suggesting the possibility of a viral transplacental transmission.
We report the case of a 34-year-old woman admitted to ICU due to severe COVID-19 infection, whose newborn died at the 37th day of life by pulmonary embolism and thrombosis of the superior vena cava.

biopsy specimens of placenta, membranes, umbilical cord, lung, thrombus, small intestine, colon, stomach, kidney, liver, spleen, esophagus and heart, have been analyzed for the presence of SARS-CoV-2 N-Protein (NP) by Immunohistochemistry. The NP staining has been analyzed using QuPath software to calculate H-Score.

The immunohistochemical examination showed positivity for SARS-CoV-2 NP in decidual placenta and at lower extent in fetal membranes (both amnion and chorion). On the contrary, the umbilical cord resulted negative. The newborn tissues analyzed showed a different positivity for SARS-CoV-2 NP: lung and heart were positive, and this latter showed a higher H-Score compared to placenta tissue ($p<0.01$, t-Student test). Similarly, also gastrointestinal tract showed the presence of the virus with a higher H-Score, in line with our recent results concerning SARS-CoV-2 bowel tropism [4]. Interestingly, also spleen and liver, known to be organs strongly associated to the gastroenteric tract, present a consistent extent of SARS-CoV-2 NP positivity ($p<0.05$, t-Student test). Conversely, kidney and thrombi showed no SARS-CoV-2 NP presence.

Since we observed SARS-CoV-2 NP positivity in different neonatal tissues, a vertical transmission is suggested, leading to possible complications in the newborn.

Acknowledgements: University of Ferrara grants: 5x1000, Crowdfunding.

References

1. Trapani, S.; Rubino, C.; Lasagni, D.; Pegoraro, F.; Resti, M.; Simonini, G.; Indolfi, G. Thromboembolic complications in children with COVID-19 and MIS-C: A narrative review. Front Pediatr 2022, 10, 944743, doi:10.3389/fped.2022.944743.
2. Flannery, D.D.; Zevallos Barboza, A.; Pfeifer, M.R.; Hudak, M.L.; Barnette, K.; Getzlaff, T.R.; Ellington, S.R.; Woodworth, K.R.; Dhudasia, M.B.; Mukhopadhyay, S.; et al. Perinatal COVID-19 maternal and neonatal outcomes at two academic birth hospitals. J Perinatol 2022, 42, 1338-1345, doi:10.1038/s41372-022-01446-x.
3. Khalil, A.; Kalafat, E.; Benlioglu, C.; O'Brien, P.; Morris, E.; Draycott, T.; Thangaratinam, S.; Le Doare, K.; Heath, P.; Ladhani, S.; et al. SARS-CoV-2 infection in pregnancy: A systematic review and meta-analysis of clinical features and pregnancy outcomes. EClinicalMedicine 2020, 25, 100446, doi:10.1016/j.eclinm.2020.100446.
4. Bortolotti, D.; Simioni, C.; Neri, L.M.; Rizzo, R.; Semprini, C.M.; Occhionorelli, S.; Laface, I.; Sanz, J.M.; Schiuma, G.; Rizzo, S.; et al. Relevance of VEGF and CD147 in different SARS-CoV-2 positive digestive tracts characterized by thrombotic damage. FASEB J 2021, 35, e21969, doi:10.1096/fj.202100821RRR.

Molecular Mechanisms Regulating BMI1 In Lung Cancer

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Non-Small Cell Lung Cancer (NSCLC) represents 85% of lung cancer cases, and is a very heterogeneous disease. Such variability hampers development of therapeutic treatments. Almost 30% of NSCLCs are driven by activating mutations in KRAS, which result in high aggressive course of disease and poor response to treatments [1]. BMI1 is a component of the epigenetic complex PRC1 [2]. It is an oncogene overexpressed in 75% of NSCLC, including the mutant KRAS molecular subtype [3]. BMI1 is positively correlated with tumor growth, invasion and metastasis [4], thus standing out as a promising therapeutic target. Pharmacological inhibition of BMI1 in NSCLC cells via a chemical compound named PTC-209, leads to growth arrest and accumulation of cells in G0 phase [4]. A microRNA-mediated mechanism of action has been hypothesized. Sequencing of microRNAs (miRs) in NSCLC cells treated with PTC-209, allowed the identification of miRs modulated by the treatment. Bioinformatics analysis identified upregulated miRNAs, the one with the highest score was miR-192. Our hypothesis is that PTC-209, by upregulating miR-192, negatively affects BMI1 expression and we tested if miR-192 downregulates BMI1 in NSCLC cells.

We generated two stable inducible mutant KRAS A549 NSCLC cell lines that allow to conditionally upregulate miR-192 or a scramble miR sequence. We established this tool that we checked by qPCR, western blot and immunofluorescence to analyze BMI1 expression levels and eventually perform functional assays.

Real Time qPCR and Western Blot analysis at 48h of miR-192 and scramble miR upregulation did not show any difference in BMI1 expression in both A549 cell lines. One- week miR-192 upregulation showed higher BMI1 mRNA expression; whereas protein expression was reduced by 50%, as compared to miR scramble expressing cells.

One-week miR-192 upregulation lead to halving BMI1 protein levels, suggesting that miR-192 may affect BMI1 mRNA translation. Identification of miR-192 as inhibitor may be a promising therapeutic strategy for decreasing oncogenic BMI1 expression. However, further studies are needed to elucidate the relationship between miR-192 and BMI1, and evaluate proliferation, migration, cell cycle and apoptotic status of cells in which miR-192 is upregulated, as compared to controls.

References

1. Maroni, G., Bassal, M. A., Krishnan, I., Fhu, C. W., Savova, V., Zilionis, R., Maymi, V. A., Pandell, N., Csizmadia, E., Zhang, J., Storti, B., Castano, J., Panella, R., Li, J., Gustafson, C. E., Fox, S., Levy, R. D., Meyerovitz, C. V., Tramontozzi, P. J., Vermilya, K., De Rienzo, A., Crucitta, S., Basseres, D. S., Weetall, M., Branstrom, A., Giorgetti, A., Ciampi, R., Del Re, M., Danesi, R., Bizzarri, R., Yang, H., Kocher, O., Klein, A. M., Welner, R. S., Bueno, R., Magli, M. C., Clohessy, J. G., Ali, A., Tenen, D. G. & Levantini, E. Identification of a targetable KRAS-mutant epithelial population in non-small cell lung cancer. Commun Biol 4, 370, doi:10.1038/s42003-021-01897-6 (2021).
2. Storti, B., Civita, S., Faraci, P., Maroni, G., Krishnan, I., Levantini, E. & Bizzarri, R. Fluorescence imaging of biochemical relationship between ubiquitinated histone 2A and Polycomb complex protein BMI1. Biophysical chemistry 253, doi:10.1016/j.bpc.2019.106225 (2019).
3. Wang, M. C., Li, C. L., Cui, J., Jiao, M., Wu, T., Jing, L. I. & Nan, K. J. BMI-1, a promising therapeutic target for human cancer. Oncology letters 10, doi:10.3892/ol.2015.3361(2015).
4. Yong, K. J., Basseres, D. S., Welner, R. S., Zhang, W. C., Yang, H., Yan, B., Alberich- Jorda, M., Zhang, J., de Figueiredo-Pontes, L. L., Battelli, C., Hetherington, C. J., Ye, M., Zhang, H., Maroni, G., O'Brien, K., Magli, M. C., Borczuk, A. C., Varticovski, L., Kocher, O., Zhang, P., Moon, Y. C., Sydorenko, N., Cao, L., Davis, T. W., Thakkar, B. M., Soo, R. A., Iwama, A., Lim, B., Halmos, B., Neuberg, D., Tenen, D. G., & Levantini, E. Targeted BMI1 inhibition impairs tumor growth in lung adenocarcinomas with low CEBPalpha expression. Sci Transl Med 8, 350ra104, doi:10.1126/scitranslmed.aad6066 (2016).

VEGFR2R1051Q and FGFR1D647N correspondent mutations exhibit pro-oncogenic effects both in vitro and in vivo

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Tyrosine kinase receptors(RTK) are frequently altered both in expression and activity in cancer. We performed a pan-cancer analysis to identify novel putative cancer driver or/and therapeutically actionable mutations of the kinase domain of different RTK[1]. To pinpoint those mutations that may be clinically relevant, we exploited the recurrence of alterations on analogous amino acid residues within the kinase domain (PK_Tyr_Ser-Thr) across different kinases as a predictor of functional impact[2]. By exploiting MutationAligner and LowMACA bioinformatics resources, we highlighted novel uncharacterized mutations in position 256 of the consensus sequences of KD. This alteration is located in the A-loop of TKD of FGFR1-4, FLT3, FLT4, PDGFRA, EGFR, VEGFR2 receptors, possibly leading to constitutive activation of the RTK[3].

Our previous results demonstrate that the substitution R1051Q in VEGFR2 leads to an increase of kinase activity and phosphorylation of receptor and supports metabolic changes. In order to evaluate if similar alterations in the tyrosine kinase domain modulate similar biological responses and druggability, we introduced the R1051Q correspondent substitution in FGFR1 (FGFR1 D647N) using the Quickchange lightning site-directed mutagenesis kit. Receptors and their mutants were expressed in HEK293T cells and assessed for ATP affinity using an ADP-glo kinase assay and phosphorylation rate through WB analysis. Cell migratory capacity and metastatic cell phenotype were assessed with both in vitro and in vivo invasion assay (i.e., CAM).

The results confirm a similar altered ATP affinity as well as a great phosphorylation for both mutant receptors. When expressed in tumoral cells both mutants lead to a strong increase in the cell migratory capacity, compared to WT receptor. Finally, it was evaluated the activity of tyrosine kinase inhibitors (TKi) including Erdafitinib, Lenvatinib, Sunitinib and Linifanib, following receptor phosphorylation by WB. Both mutated receptors exhibit higher sensitivity to TKi.

Our data confirm our previous hypotheses concerning the biological effect of these mutations. Future studies will allow to extend this knowledge to other significant mutations in the same hotspot position. This concept is particularly important for therapeutically actionable mutations. Indeed, PD-based analyses have the potential to accelerate the choice of patient-specific targeted drugs.

References

1. Grillo et al, Cancer Letters (2021), 10.1016/j.canlet.2020.09.027.
2. Grillo et al, Cancer Letters (2021), 10.1016/j.canlet.2021.03.007.
3. Grillo et al, Molecular Basis of disease (2021), 10.1016/j.bbadis.2021.166313

Cancer stem-like cells: a new therapeutic target for the treatment of uveal melanoma

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Cancer stem-like cells (CSCs) are a subpopulation of tumor cells responsible for tumor initiation, metastasis, chemoresistance, and relapse [1]. Recently, CSCs have been identified in Uveal Melanoma (UM), which is the most common primary tumor of the eye [2, 3]. UM is highly resistant to systemic chemotherapy and effective therapies aimed at improving overall survival of patients are eagerly required [4].

Herein, taking advantage from the pan Fibroblast Growth Factor (FGF)-trap molecule NSC12 [5], we singled out and analyzed a UM-CSC subset with marked stem-like properties. A hierarchical clustering of gene expression was performed on data publicly available on The Cancer Genome Atlas (TCGA).

Our data show a significant reduction of cell proliferation following the inhibition of the FGF/FGF receptor (FGFR) signaling. Moreover, NSC12 has been exploited to unmask an FGF-sensitive UM population characterized by enhanced aldehyde dehydrogenase (ALDH) activity and tumor-sphere formation capacity [Panel A], as well as by increased expression of numerous stemness-related transcription factors. Moreover, FGF inhibition deeply affected UM-CSC survival in vivo in a chorioallatoic membrane (CAM) tumor graft assay, resulting in the reduction of tumor growth. At clinical level, hierarchical clustering of TCGA gene expression data revealed a strong correlation between FGFs/FGFRs and stemness-related genes, allowing the identification of three distinct clusters in patients characterized by different clinical outcomes.

Our findings support the evidence that the FGF/FGFR axis represents a master regulator of cancer stemness in primary UM tumors, pointing to anti-FGF treatments as a novel therapeutic strategy to hit the CSC component in UM.

References

1. Yadav, A.K. and N.S. Desai, Cancer Stem Cells: Acquisition, Characteristics, Therapeutic Implications, Targeting Strategies and Future Prospects. Stem Cell Rev Rep, 2019. 15(3): p. 331-355.
2. Kalirai, H., B.E. Damato, and S.E. Coupland, Uveal melanoma cell lines contain stem-like cells that self-renew, produce differentiated progeny, and survive chemotherapy. Invest Ophthalmol Vis Sci, 2011. 52(11): p. 8458-66.
3. Chen, Y.N., Y. Li, and W.B. Wei, Research Progress of Cancer Stem Cells in Uveal Melanoma. Onco Targets Ther, 2020. 13: p. 12243-12252.
4. Carvajal, R.D., et al., Metastatic disease from uveal melanoma: treatment options and future prospects. Br J Ophthalmol, 2017. 101(1): p. 38-44.
5. Ronca, R., et al., Long-Pentraxin 3 Derivative as a Small-Molecule FGF Trap for Cancer Therapy. Cancer Cell, 2015. 28(2): p. 225-39.

Primary monocytes as carriers for the oncolytic virotherapy of glioblastoma

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Oncolytic viruses (OVs) are therapeutics which combine cancer cell-killing activity, immunotherapy and gene therapy. Cancer cells are more susceptible than healthy cells to viral replication, thus OVs were derived by attenuation of viruses including herpes simplex virus type 1 (HSV-1). Oncolytic HSV-1 (oHSV1) talimogene laherparepvec was approved for the treatment of unresectable melanoma in the USA and the EU. OV research focuses on increasing effectiveness in different tumors, including glioblastoma (GBM), the most frequent primary tumor of the brain, which has a very poor prognosis. The most employed delivery method is intratumoral administration, both in preclinical and clinical studies, but a strategy to achieve systemic delivery involves using carrier cells infected ex vivo and injected, shielding the OV from the immune system. Here we propose a novel approach, i.e. the use of circulating monocytes as carrier cells for oHSV1. Human monocytes can be infected by HSV-1 but are relatively resistant to infection. In many tumors, including GBM, circulating monocytes are precursors of tumor-associated macrophages (TAMs), and are actively recruited by cancer cells.

Bacterial artificial chromosome (BAC) mutagenesis was used to construct oHSV-1 vectors with the same genetic deletions as talimogene laherparepvec, carrying reporter genes (enhanced green fluorescent protein or EGFP) or therapeutic genes (human interleukin 12, hIL-12), or to insert the target sequence of the neuron-specific microRNA mir124 downstream of the essential UL29 gene. Primary human monocytes (PHM) fromhealthy blood donors were isolated by Ficoll gradient centrifugation and purified by adhesion.

oHSV-EGFP replicated and showed cytotoxicity in GBM cell lines of human (U87-MG and LN-229) and murine origin (GL261, CT2A). PHM infected with oHSV-EGFP or oHSV-hIL12 could transfer the infection to human GBM cells in vitro. Cell culture medium conditioned by human GBM cells was chemotactic for PHM.

In vitro data indicate that PHM are efficient carriers for oHSV1 vectors to treat GBM. This capacity is maintained also for vectors expressing therapeutic genes such as IL-12. The novel oHSV-mir124 is attenuated in human brain organoids but not in human and murine GBM cells, thus it can provide the basis for the development of future vectors.

References

- Kaufman, H.L.; Kohlhapp, F.J.; Zloza, A. Oncolytic Viruses: A New Class of Immunotherapy Drugs. Nature Reviews Drug Discovery 2015, 14, 642–662.
- Conry, R.M.; Westbrook, B.; McKee, S.; Norwood, T.G. Talimogene Laherparepvec: First in Class Oncolytic Virotherapy. Human Vaccines and Immunotherapeutics 2018, 14, 839–846.
- Tykocki, T.; Eltayeb, M. Ten-Year Survival in Glioblastoma. A Systematic Review. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia 2018, 54, 7–13, doi:10.1016/J.JOCN.2018.05.002
- Reale, A.; Calistri, A.; Altomonte, J. Giving Oncolytic Viruses a Free Ride: Carrier Cells for Oncolytic Virotherapy. Pharmaceutics 2021, 13, 2192, doi:10.3390/PHARMACEUTICS13122192

A new computational framework for predicting cancer drug response

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Predicting the clinical response of therapeutic agents is a major challenge in cancer treatment. Unfortunately, establishing accurate and robust methods in such a context is difficult. Most approaches exploit only clinical data and gene expression information without considering other resources, such as pathways, capable of shedding light on the comprehension of such phenomena.

We propose a workflow consisting of 6 main steps. First, TCGA-curated drug response clinical data [1] are retrieved and preprocessed as described in [2]. Secondly, each cancer-drug dataset is divided into train/test sets. Then, we identify differentially expressed genes in each sample by computing the 25th and 75th percentile of the gene distribution in normal samples. Next, for each gene, we build a contingency matrix including the number of responders and non-responders samples having that gene greater than 75 percentile or less than 25 percentile. Finally, a chi-square test is applied to select the enrichment genes list. Such a list is used as input to the PHENSIM [3] simulator, together with expression data for each patient, to get the perturbation of genes. Finally, the PHENSIM simulator results are used to train a learning model capable of predicting drug response.

We analyzed the performance of drug response prediction in terms of Accuracy, Precision, Recall, and F-measure on TCGA-BLCA patients treated with Cisplatin and Gemcitabine. Both endpoint/pathway perturbation and activity score were independently used for the model's training. Then, the counts matrix, including all the genes and a subset containing the significant genes selected in step 4, was used for the model training. The drug response prediction using endpoint and pathway perturbation outperformed the other analysis, yielding an accuracy of 85.71% and 88.88%, respectively.

We presented a new drug response prediction method and tested it on TCGA-BLCA patients. The results obtained support future analysis in different TCGA patients and datasets.

References

1. Ding Z, Zu S, Gu J. Evaluating the molecule-based prediction of clinical drug responses in cancer. Bioinformatics. 2016;32: 2891–2895.
2. Hermida LC, Gertz EM, Ruppin E. Predicting cancer prognosis and drug response from the tumor microbiome. Nat Commun. 2022;13: 2896.
3. Alaimo S, Rapicavoli RV, Marceca GP, La Ferlita A, Serebrennikova OB, Tsichlis PN, et al. PHENSIM: Phenotype Simulator. PLoS Comput Biol. 2021;17: e1009069.

Intracranial injection of CIITA-driven MHC-II positive glioblastoma cells induce an effective anti-tumor immune response in vivo: implication for new therapeutic strategies against the glioblastoma

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Glioblastoma (GBM) is the most aggressive brain tumor with dismal prognosis despite standard of care including surgery, radiotherapy, and chemotherapy. The recent success of immunotherapy for other aggressive cancers has given new hope for extending it to GBM as well. However, the main challenges of immunotherapy for GBM are the blood brain barrier that strongly limits access to the intracranial location and relative immunosuppressive tumor microenvironment observed in brain tumor tissue. Our previous studies showed that CIITA-induced MHC class II expression in tumor cells of distinct histological origin renders these cells surrogate antigen presenting cells of their own tumor antigens, leads to a T helper 1 (Th1) polarization of the tumor microenvironment, tumor rejection and acquisition of specific anti-tumor memory. Here we report the unprecedented positive results of our approach to GBM.

Immunocompetent C57BL/6 mice were implanted intracranially (i.c.) in one hemisphere with the GL261 glioma parental tumor cells (GL261pc) or the same cells transfected with CIITA (GL261-CIITA). At 3,7,14 and 21 days post-injection (p.i.) mice were sacrificed and the brains were analyzed for the presence of the tumor and the tumor infiltrating cells by immunohistochemistry. To assess acquisition of protective anti-tumor immunity GL261-CIITA injected mice at 21 days post injection were injected in the opposite hemisphere with GL261pc and sacrificed 21 days after the last injection. The presence of both parental and CIITA tumor and the cellular infiltrate was assessed as specified above.

GL261-CIITA tumors are rejected or significantly reduced in their growth compared to GL261pc tumors. This correlates with a cellular infiltrate mostly represented in GL261-CIITA injected mice by both CD4 and CD8 T lymphocytes. Preimplantation of GL261-CIITA tumor cells results in complete rejection or strong growth retardation of GL261pc tumor cells in the opposite hemisphere.

GL261-CIITA cells are potent stimulators of an adaptive immune response in vivo, able to protect the mouse from tumor take. GL261-CIITA tumors vaccination prevented or strongly reduced GL261pc tumor growth in the opposite hemisphere, suggesting that an effective intracranial anti-tumor immune response can be established as a consequence of Th cell restricted-recognition of GBM-specific antigens.

References

- Germain RN, Margulies DH. The biochemistry and cellular biology of antigen processing and presentation. Annu Rev Immunol 1993; 11:403-50; PMID:8476568.;http://dx.doi.org/10.1146/annurev.iy.11.040193.02155
- Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S,Soares H, Brimnes MK, Moltedo B, Moran TM, Steinman RM. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. J Exp Med 2004; 199:815-24; PMID:15024047; http://dx.doi.org/10.1084/jem.20032220
- Steinman RM. Dendritic cells: versatile controllers of the immune system. Nature Med 2007; 13:1155-9; PMID:17917664; http://dx.doi.org/10.038/nm643

- Pardoll DM, Topalian SL. The role of CD4C T cell responses in anti-tumor immunity. Curr Opin Immunol 1998; 10:588-94;PMID:9794842; http://dx.doi.org/10.1016/S0952- 7915(98)80228-8
- Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll DM,Levitsky H. The central role of CD4C T cells in the antitumor immune response. J Exp Med 1998; 188:2357-68; PMID:9858522; http://dx.doi.org/10.1084/jem.188.12.2357
- Frangione, V., Mortara, L., Castellani, P., De Lerma Barbaro, A., andAccolla, R. S. (2010) CIITA-driven MHC-II positive tumor cells: Preventive vaccines and superior generators of antitumor CD4+ T lymphocytes for immunotherapy. Int. J. Cancer 127, 1614 – 1624
- Meazza, R., Comes, A., Orengo, A. M., Ferrini, S., and Accolla, R. S. (2003) Tumor rejection by gene transfer of the MHC class II transactivator in murine mammary adenocarcinoma cells. Eur. J. Immunol. 33, 1183 – 1192
- Accolla RS, Jotterand-Bellomo M, Scarpellino L, Maffei A, Carra G, Guardiola J. aIr-1, a newly found locus on mouse chromosome 16 encoding a trans-acting activator factor for MHC class II gene expression. The Journal of experimental medicine 1986;164(1):369-74.
- Mortara, L., Castellani, P., Meazza, R., Tosi, G., De Lerma Barbaro, A.,Procopio, F. A., Comes, A., Zardi, L., Ferrini, S., and Accolla, R. S. (2006)CIITA-induced MHC class II expression in mammary adenocarcinoma leads to a Th1 polarization of the tumor microenvironment, tumor rejection, and specific antitumor memory. Clin. Cancer Res. 12, 3435 – 3443
- Accolla RS, Tosi G. Adequate antigen availability: a key issue for novel approaches to tumor vaccination and tumor immunotherapy J Neuroimmune Pharmacol.2013;8:28-36. DOI:10.1007/s11481-012-9423-7.
- Accolla RS, Lombardo L, Abdallah R, Raval G, Forlani G, Tosi G. Boosting the MHC class II-restricted tumor antigen presentation to CD4+ T helper cells: a critical issue for triggering protective immunity and re-orienting the tumor microenvironment toward an anti-tumor state. Frontiers Oncol. 2014;4:32. DOI: 10.3389/fonc.2014.00032. doi: 10.3389/fonc.2014.00032.
- Bou Nasser Eddine F, Forlani G, Lombardo L, Tedeschi A, Tosi G, Accolla RS. CIITA-driven MHC class II expressing tumor cells can efficiently prime naive CD4+ TH cells in vivo and vaccinate the host against parental MHC-II-negative tumor cells. OncoImmunology. 2017;6(1):e1261777. doi: 10.1080/2162402X.2016.1261777.
- Szatmári T, Lumniczky K, Désaknai S, Trajcevski S, Hídvégi EJ, Hamada H, et al. Detailed characterization of the mouse glioma 261 tumor model for experimental glioblastoma therapy. Cancer Science. 2006;97(6):546-53. doi: 10.1111/j.1349-7006.2006.00208.x.
- Ausman JI, FShapiro WR, Rall DP. Studies on the chemotherapy of experimental Bbrain tumors: development of an experimental model. Cancer Res. 1970;30:2394-400.
- Engelhardt B, Vajkoczy P, Weller RO. The movers and shapers in immune privilege of the CNS. Nat Immunol. 2017;18(2):123-31. doi: 10.1038/ni.3666.
- Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):41-53.
- Davies DC. Blood–brain barrier breakdown in septic encephalopathy and brain tumours*. Journal of Anatomy. 2002;200(6):639-46. doi: 10.1046/j.1469-7580.2002.00065.x.
- Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. The Journal of Experimental Medicine. 2015;212(7):991-9. doi: 10.1084/jem.20142290.
- Louveau A, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. Trends in Immunology. 2015;36(10):569-77. doi: 10.1016/j.it.2015.08.006.
- Reardon DA, Gokhale PC, Klein SR, Ligon KL, Rodig SJ, Ramkissoon SH, et al. Glioblastoma Eradication Following Immune Checkpoint Blockade in an Orthotopic, Immunocompetent Model. Cancer Immunology Research. 2016;4(2):124-35. doi: 10.1158/2326-6066.cir-15-0151.
- Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell. Oct 10 2013;155(2):462-477.
- Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008 Jul 31;359(5):492-507.
- Miranda W.Yu, Daniela F.Quail Immunotherapy for Glioblastoma: Current Progress and Challenges, Front. Immunol., 13 May 2021 | https://doi.org/10.3389/fimmu.2021.676301
- Louis, D. N.; Ohgaki, H.; Wiestler, O. D.; Cavenee, W. K.; Burger, P. C.; Jouvet, A.; Scheithauer, B. W.; Kleihues, P. The 2007 WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol 2007, 114, 97-109.

Identification of prognostic gene markers using transcriptome analysis in hypoxic mesothelioma

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Malignant Mesothelioma (MM) is a type of cancer arising from the mesothelium, a thin layer of cells, lining internal organs. Primarily caused by asbestos exposure, over 30,000 people are diagnosed with MM annually. Therapeutic measures, which include chemo- and radiotherapy, and surgery in some cases, poorly increase life expectancy and there is no permanent cure for this disease [1]. Therefore, early detection and prognostic prediction are important to improve the survival in MM patients. Pleural MM induces respiratory distress leading to hypoxia. Hypoxia, in turn, leads to accumulation of adenosine which favors tumor progression by suppressing T cell mediated immune response [2].

Targeting adenosine receptors recently emerged as a promising anti-tumoral strategy. To mimic active hypoxia, mesothelioma cell line REN was treated with NECA [an adenosine receptor agonist] for 2 hours which effectively induced intracellular CREB phosphorylation [3]. Then, by whole transcriptomics analysis, we studied expression pattern of NECA-treated cells compared to control. We found a total of 199 differentially expressed genes (DEGs) and non-coding RNAs after NECA treatment. Survival related DEGs and lncRNAs associated with mesothelioma patient prognosis were identified in silico using GEPIA2 and lncSEA, respectively. Further, protein coding genes and lncRNAs were shortlisted based on significant p-value (<0.05) in differential expression and GEPIA2 overall survival (OS) analysis for experimental validation. In KEGG Pathway & Gene Ontology analysis we have found significant regulation of oxygen transport and ribosome complex formation confirming a role of adenosine in the hypoxia process. RT-PCR analysis confirmed increased expression of CNIH2 (Cornichon Family AMPA Receptor Auxiliary Protein 2) and decreased expression of GLN1 (G protein nucleolar 1) in NECA treated mesothelioma cells. These protein coding genes are associated to significant changes in OS of MM patients suggesting a role for these proteins as both prognostic and therapeutic targets.

References

1. Mesothelioma. http://www.medicinenet.com/mesothelioma/article.htm.
2. Sorrentino C and Morello S.; Role of adenosine in tumor progression: focus on A2B receptor as potential therapeutic target. J Cancer Metastasis Treat 2017; 3:127-38. http://dx.doi.org/10.20517/2394-4722.2017.29
3. Leonard MO et al. Hypoxia selectively activates the CREB family of transcription factors in the in vivo lung. Am J Respir Crit Care Med. 2008 Nov 1;178(9):977-83. doi:10.1164/rccm.200712-1890OC. Epub 2008 Aug 8

The endogenous HBZ interactome in ATL leukemic cells reveals an unprecedented complexity of host interacting partners involved in RNA splicing

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Adult T-cell leukemia/lymphoma (ATL) is a T-cell lymphoproliferative neoplasm caused by the human T-cell leukemia virus type 1 (HTLV-1). Two viral proteins, Tax-1 and HBZ play important roles in HTLV-1 infectivity and in HTLV-1-associated pathologies by altering key pathways of cell homeostasis. However, the molecular mechanisms through which the two viral proteins, particularly HBZ, induce and/or sustain the oncogenic process are still largely elusive.

Previous results suggested that HBZ interaction with nuclear factors may alter cell cycle and cell proliferation. To have a more complete picture of the HBZ interactions, we investigated in detail the endogenous HBZ interactome in leukemic cells by immunoprecipitating the HBZ-interacting complexes of ATL-2 leukemic cells, followed by tandem mass spectrometry analyses. RNA seq analysis was performed to decipher the differential gene expression and splicing modifications related to HTLV-1. Here we compared ATL-2 with MOLT-4, a non HTLV-1 derived leukemic T cell line and further compared with HBZ-induced modifications in an isogenic system composed by Jurkat T cells and stably HBZ transfected Jurkat derivatives.

The endogenous HBZ interactome of ATL-2 cells identified 249 interactors covering three main clusters corresponding to protein families mainly involved in mRNA splicing, nonsense-mediated RNA decay (NMD) and JAK-STAT signaling pathway. Here we analysed in detail the cluster involved in RNA splicing. RNAseq analysis showed that HBZ specifically altered the transcription of many genes, including crucial oncogenes, by affecting different splicing events. Consistently, the two RNA helicases, members of the RNA splicing family, DDX5 and its paralog DDX17, recently shown to be involved in alternative splicing of cellular genes after NF-κB activation by HTLV-1 Tax-1, interacted and partially co-localized with HBZ. For the first time, a complete picture of the endogenous HBZ interactome was elucidated.

The wide interaction of HBZ with molecules involved in RNA splicing and the subsequent transcriptome alteration strongly suggests an unprecedented complex role of the viral oncogene in the establishment of the leukemic state.

References

- Shallak M, Alberio T, Fasano M, Monti M, Iacobucci I, Ladet J, Mortreux F, Accolla RS, Forlani G. The endogenous HBZ interactome in ATL leukemic cells reveals an unprecedented complexity of host interacting partners involved in RNA splicing. Front Immunol. 2022 Aug 1;13:939863. doi: 10.3389/fimmu.2022.939863. PMID: 35979358; PMCID: PMC9376625.

Classification of cancer mutation clonality and zygosity from target sequencing panels

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High-throughput bulk targeted sequencing panels of hundreds of genes are clinical standards to retrieve actionable tumour mutations for precision medicine initiatives. One intrinsic limitation of these assays is the lack of strong statistical signals to determine mutation clonality and copy number profiles for the panel genes. We leveraged on ideas from tumour subclonal deconvolution (Caravagna et al. 2020) and copy number calling (Househam et al. 2022) in order to define a statistical framework that can classify tumour somatic variants without having to resort on advanced bioinformatics pipelines.

Our framework use panel read counts data to determine the clonality status of tumour mutations while estimating their zygosity. We trained the parameters of our model using whole-genome and whole-exome sequencing datasets where clonality and zygosity states are known, reaching high accuracy levels. We used our model to classify oncogenes and tumour suppressor genes in thousands of samples from the recent MSK MetTropism cohort, determining a wide portrait of potential early alterations, complete suppressor inactivation and oncogene activation events across human cancers. As recently shown (Spurr et al. 2022) aneuploidy plays a potential role for the efficacy of immunotherapy among tumors. We defined genotypes as combinations of clonal mutations and copy number alterations on multiple genes, extracted the recurrent ones and measured their contribution to patient survival, along with other available clinical features.

We implemented our framework in a software package (TAPACLOTH, available at https://github.com/caravagnalab/TAPACLOTH) that can be readily used downstream of targeted sequencing assays routinely performed in clinical infrastructures.

References:

- Caravagna, Giulio, Timon Heide, Marc J. Williams, Luis Zapata, Daniel Nichol, Ketevan Chkhaidze, William Cross, et al. 2020. “Subclonal Reconstruction of Tumors by Using Machine Learning and Population Genetics.” Nature Genetics 52 (9): 898–907. https://doi.org/10.1038/s41588-020-0675-5.
- Househam, Jacob, Riccardo Bergamin, Salvatore Milite, Nicola Calonaci, Alice Antonello, Marc J. Williams, William C. H. Cross, and Giulio Caravagna. 2022. “Integrated Quality Control of Allele-Specific Copy Numbers, Mutations and Tumour Purity from Cancer Whole Genome Sequencing Assays.” bioRxiv. https://doi.org/10.1101/2021.02.13.429885.
- purr, L.F., Weichselbaum, R.R. and Pitroda, S.P. 2022 "Tumor aneuploidy predicts survival following immunotherapy across multiple cancers". Nature Genetics https://doi.org/10.1038/s41588-022-01235-4.

Tumor recognition and acquisition of protecting adaptive anti-tumor immune response in vivo against CIITA-driven MHC class II expressing Oral Squamous Cell Carcinoma Cells 6

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We have previously demonstrated that cells from MHC class II-negative solid tumors can function as surrogate Antigen Presenting Cells (APCs) for their tumor antigens, provided they optimally express MHC class II (MHC-II) molecules as a function of genetic transfer of the MHC class II transactivator CIITA. CIITA positive tumors became potent stimulators of a protective adaptive antitumor immune response, triggered by CD4+ T helper (Th) cells and mediated by both Th cells and tumor specific CD8+ cytolytic (CTL). Here we extend our approach to Oral Squamous Cell Carcinoma (OSCC), the most common malignant neoplasm of the oral cavity for which the prognosis is poor due to a high rate of locoregional recurrence and development of distant metastasis.

Syngeneic C57BL/6 mice were injected subcutaneously (s.c.) with either parental Mouse Oral Cancer 2 parental (MOC2pc) or MOC2-CIITA tumor cells and tumor growth was checked at least twice a week. Mice which have rejected MOC2-CIITA tumor cells were challenged s.c. with MOC2pc. The size for the tumor was measured weekly. For Adoptive Cells Transfer (ACT) experiments, mice were injected s.c. MOC2 cells plus total naïve splenocytes, or total immune splenocytes, or immune CD4+ or CD8+ lymphocytes previously isolated from spleens of mice vaccinated with MOC2-CIITA tumor cells and challenged with MOC2pc that showed no tumor growth.

CIITA-driven MHC-II+ MOC2 tumor cells were rejected or strongly retarded in their growth in vivo. When challenged with MOCpc, these animals strongly delayed tumor growth, indicating the acquisition of an anamnestic response. ACT experiments showed that total spleen cells from tumor protected mice, as well as CD8+ and, more importantly, CD4+ spleen cells significantly protected from MOC2pc tumor take, demonstrating the ability of CIITA-transfected tumor cells to stimulate an adaptive immunity based on the triggering of tumor specific Th and CTL cells.

These results validate our vaccination approach also in OSCC emphasizing the importance of the expression of MHC-II molecules driven by CIITA, to render tumor cells surrogate APC for their tumor antigens in vivo and thus open the way to characterize the specific antigenic peptides valuable for possible new anti-tumor vaccines.

3. References

- 1. Accolla RS, Scarpellino L, Carra G, Guardiola J. Trans-acting element(s) operating across species barriers positively regulate the expression of major histocompatibility complex class II genes. J Exp Med. 1985;162:1117-33.
- 2. Accolla RS, Jotterand-Bellomo M, Scarpellino L, Maffei A, Carra G, Guardiola J. Air-1, a newly found locus on mouse chromosome 16 encoding a trans-acting activator factor for MHC class II gene expression. J Exp Med. 1986;164:369-74.
- 3. Steimle V, Otten LA, Zufferey M, and Mach B. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or Bare Lymphocyte Syndrome). Cell. 1993;75 135-46.
- 4. Meazza R, Comes A, Orengo AM, Ferrini S, and Accolla RS. Tumor rejection by gene transfer of the MHC class II transactivator in murine mammary adenocarcinoma cells. Eur J Immunol. 2003;33:1183-92. DOI: 10.58/078-0432.CCR-06-165.
- 5. Mortara L, Castellani P, Meazza R, Tosi G, De Lerma Barbaro A, Procopio FA, et al. CIITA induced MHC class II expression in mammary adenocarcinoma leads to a Th1 polarization of the tumor microenvironment, tumor rejection, and specific antitumor memory. Clin Cancer Res. 2006;12:3435-43. DOI: 10.1158/078-0432.CCR-06-165.
- 6. Frangione V, Mortara L, Castellani P, De Lerma Barbaro A, Accolla RS. CIITA-driven MHC II positive tumor cells: preventive vaccines and superior generators of anti-tumor CD4+ T lymphocytes for immunotherapy. Int J Cancer. 2010;127:1614-24. DOI: 10.002/ijc.25183. 7.Bou Nasser Eddine F, Forlani G, Lombardo L, Tedeschi A, Tosi G, Accolla RS. CIITA-driven
- MHC class II expressing tumor cells can efficiently prime naive CD4+ TH cells in vivo and vaccinate the host against parental MHC-II-negative tumor cells. OncoImmunology. 2017;6(1):e1261777. doi: 10.1080/2162402X.2016.1261777.

Outer Membrane Vesicles Derived from Klebsiella pneumoniae Influence the miRNA Expression Profile in Human Bronchial Epithelial BEAS-2B Cells

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Klebsiella pneumoniae is an opportunistic pathogen that causes nosocomial and community-acquired infections. K. pneumoniae releases outer membrane vesicles (OMVs). OMVs are rounded nanostructures released during their growth by Gram-negative bacteria. These vesicles are a vehicle for the transport of virulence factors to host cells, causing cell damage. Our previous studies have shown changes in human bronchial epithelial cells (BEAS-2B) gene expression, after treatment with K. pneumoniae-OMVs (1). These variations in gene expression could be regulated through microRNAs (miRNAs), which participate in several biological mechanisms. Little is known about the function of miRNAs in the BEAS-2B cells after OMVs interaction. Therefore, the aim of our study was to evaluate the miRNA expression profiles in BEAS-2B cells during infection with OMVs derived from standard and clinical K. pneumoniae strains.

OMVs from K. pneumoniae ATCC 10031 and clinical isolates were purified using serial filtration and ultracentrifugation. OMVs were quantified using Bradford assays. The vesicle diameter size and polydispersity index were defined by Dynamic Light Scattering (DLS). BEAS 2B cells were treated with 5µg /mL of OMVs for 6 hours and after miRNA extractions were performed. The expression profiles of microRNA were performed using a 384-well TaqMan Human MicroRNA array. TargetScan, DIANA-microT-CDS and miRTarBase were exploited to predict the target genes of the miRNA dataset. The Metascape software was used for Gene Ontology enrichment analysis. Transcripts levels of four miRNAs were measured by RT-qPCR.

All vesicles were analyzed in terms of diameter and size distribution. DLS analysis had shown that OMVs from standard strain were characterized by a smaller diameter and slightly heterogeneous size distribution compared to the OMVs from clinical K. pneumoniae. In addition, clinical strains produced more OMVs than the standard ones. Microarray analysis and RT- qPCR identified the dysregulation of miR-223, hsa-miR-21, hsa-miR-25 and hsa-let-7g miRNAs sequences. Target gene prediction revealed an essential role of these miRNAs in the regulation of host immune responses affecting NF-κB (miR-223), TLR4 (hsa miR-21), cytokine (hsa-miR-25) and IL- 6 (hsa-let-7g miRNA) signalling pathways.

Our results suggested an important role of OMVs in the inflammatory response via miRNAs regulation.

References

- Martora, F.; Pinto, F.; Folliero, V.; Cammarota, M.; Dell'Annunziata, F.; Squillaci, G.; Galdiero, M.; Morana, A.; Schiraldi, C.; Giovane, A.; et al. Isolation, characterization and analysis of pro-inflammatory potential of Klebsiella pneumoniae outer membrane vesicles. Microb. Pathog. 2019, 136, 103719.

Influence of HLA-C genotype on HIV-1 progression

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Understanding the differences in HIV-1 progression is an important concern that needs to be investigated. More knowledge about the influence of host genetic factors is required to better understand the different phenotypes of HIV-1 progression. Previous studies showed that HLA-C variants that are less expressed and less stably bound to β2-microglobulin/peptide complex are associated with poor HIV-1 control and increased HIV-1 infectivity [1,2]. Therefore, we investigated whether there is a correlation between different stages of HIV-1 progression and the presence of specific HLA-C allotypes.

HLA-C genotyping was performed through an allele-specific PCR approach by analysing a treatment-naïve cohort of 96 HIV-1 infected patients from USA, Canada, and Brazil. HIV-1- positive subjects were classified according to their disease progression status as Progressors (n = 48), Long-Term Non-Progressors (n = 37) and Elite Controllers (n = 11). HLA-C variants were ranked as stable or unstable based on their binding stability to β2- microglobulin/peptide complex [3,4].

Our results showed a significant correlation between the presence of two or one HLA-C unstable variants and a more rapid HIV-1 progression (p-value: 0.0078, p-value: 0.0143, respectively). These findings strongly suggest a link between unstable HLA-C variants at both genotype and at alleles level and rapid HIV-1 progression.

This work provides further insights into the impact of host genetic factors on HIV-1 progression.

References

- Apps, R.; Qi, Y.; Carlson, J.M.; Chen, H.; Gao, X.; Thomas, R.; Yuki, Y.; Del Prete, G.Q.; Goulder, P.; Brumme, Z.L.; et al. Influence of HLA-C Expression Level on HIV Control. Science 2013, 340, 87, doi:10.1126/SCIENCE.1232685.
- Parolini, F.; Biswas, P.; Serena, M.; Sironi, F.; Muraro, V.; Guizzardi, E.; Cazzoletti, L.; Scupoli, M.T.; Gibellini, D.; Ugolotti, E.; et al. Stability and Expression Levels of HLA-C on the Cell Membrane Modulate HIV-1 Infectivity. J. Virol. 2018, 92, 1–17, doi:10.1128/jvi.01711-17.
- Sibilio, L.; Martayan, A.; Setini, A.; Monaco, E. Lo; Tremante, E.; Butler, R.H.; Giacomini, P. A Single Bottleneck in HLA-C Assembly. J. Biol. Chem. 2008, 283, 1267–1274, doi:10.1074/jbc.M708068200.
- Zipeto, D.; Serena, M.; Mutascio, S.; Parolini, F.; Diani, E.; Guizzardi, E.; Muraro, V.; Lattuada, E.; Rizzardo, S.; Malena, M.; et al. HIV-1-Associated Neurocognitive Disorders: Is HLA-C Binding Stability to B2-Microglobulin a Missing Piece of the Pathogenetic Puzzle? Front. Neurol. 2018, 9, 791, doi:10.3389/FNEUR.2018.00791.

BioVRPi: can bioinformatics and genomics become pocket-sized and affordable to everyone?

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The current socio-economic and environmental situation underline the urgency of low-cost and sustainable computational alternatives. Moreover, in recent years the bioinformatics community has shown renewed interest for Raspberry Pi (RPi) applications in teaching and research projects. In the context of the BioVRPi project [1,2] - which aims to develop a low-cost and tested bioinformatic environment - we propose an exploratory cross-platform benchmarking of multi-organism genomic analyses.

Benchmark of indexing and alignment processes was carried out on RPi 4, laptop, and desktop. Performance assessment was evaluated on SARS-CoV-2, Escherichia coli and Caenorhabditis elegans genomes. To minimize variability and biases, sample reads were generated in silico (30X coverage) from their respective reference genomes using ART Illumina v2.5.8 [3]. Indexing and alignment were performed with 3 tools: BWA v0.7.17-r1188 [4], Bowtie2 v2.4.5 [5], and Minimap2 v2.17 [6,7], scaling from 1 to 4 threads.Benchmarking was evaluated using Hyperfine v1.13.0 [8].

We performed a cross-platform benchmark of multi-organism genomic indexing and short reads alignment to evaluate RPi as a viable alternative to common bioinformatic devices. The computational times for indexing and alignment are reported in Table 1. As regards indexing, we observed comparable runtimes among RPi and other platforms using BWA and Bowtie2 for SARS-CoV-2 and E. coli, whereas Minimap2 indexing showed an increase of one order of magnitude in runtimes for RPi and the fastest running times overall. As regards the alignment process, we observed consistency in runtimes differences across all organisms and tools. Overall, Minimap2 performances proved to be the fastest whereas Bowtie2 displayed poor performances across all platforms, exacerbating its inefficiency on RPi. Benchmarking analyses considered multi-threading up to 4 threads, the maximum available on RPi: indexing didn't show significant improvements; conversely, alignment showed the best performances using multi-threading as expected.

RPi proved to be a viable alternative to perform genomic data analysis on different organisms and turned out to be particularly efficient for microorganisms. Further advances and tools optimization for RPi ARM architecture will lead to a greater scalability for complex organisms and will be carried out by the BioVRPi project in future exploratory analyses.

References

1. Treccani M, Veschetti L, Locatelli E, De Tomi E, Leoni B, Gallinaro M, Dagnogo D, Moron Dalla Tor L, Patuzzo C, Malerba G. Pocket-sized genomics - and transcriptomics analyses: a look at the newborn BioVRPi project [version 1; not peer reviewed]. F1000Research 2021, 10(ISCB Comm J):1181 (poster) doi: 10.7490/f1000research.1118855.1.
2. Treccani M, Veschetti L, Malerba G. The BioVRPi project: a valuable and sustainable alternative for genomic analysis [version 1; not peer reviewed]. F1000Research 2022, 11:1310 (poster) doi: 10.7490/f1000research.1119195.1.
3. Huang W, Li L, Myers JR, Marth GT. ART: a next-generation sequencing read simulator. Bioinformatics. 2012 Feb 15;28(4):593-4. doi: 10.1093/bioinformatics/btr708.
4. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15;25(14):1754-60. doi: 10.1093/bioinformatics/btp324.
5. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012 Mar 4;9(4):357-9. doi: 10.1038/nmeth.1923.
6. Li H. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018 Sep 15;34(18):3094-3100. doi: 10.1093/bioinformatics/bty191.
7. Li H. New strategies to improve minimap2 alignment accuracy. Bioinformatics. 2021 Oct 8;37(23):4572–4. doi: 10.1093/bioinformatics/btab705.
8. Peter, D. (2022). hyperfine (Version 1.15.0) [Computer software]. https://github.com/sharkdp/hyperfine

Integrated quality control of allele-specific copy numbers, mutations and tumour purity from cancer whole genome sequencing assays

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Cancer genomes harbour multiple types of somatic mutations which can be retrieved from DNA sequencing. The most common are single nucleotide variants (SNVs) and copy number alterations (CNAs), the calling of the latter requiring an accurate estimate of tumour purity. Despite a clear connection between the variant allele frequency (VAF) of SNVs and allelic copy number, SNVs and CNA calling are performed as decoupled analysis, yielding, in some cases, to inconsistent results. To tackle this issue, we developed CNAqc, a quantitative framework to QC allele-specific CNAs, tumour purity and SNVs from bulk sequencing.

The foundational idea of CNAqc is to check whether the VAF distribution of SNVs sitting on segments with the same declared copy number state is in agreement with the expected one. Expected VAF peaks positions are computed on pooled collections of SNVs sitting on segments with the same copy number state, accounting for sample purity; these theoretical positions are then compared to those detected in data through a kernel-based or a binomial mixture-based approach. The distance between the two is used to compute a quality score over the different copy states found in the genome and a PASS/FAIL flag is assigned to each of them and on a sample-level.

CNAqc is implemented as an open source R package to be used after variant calling from either whole genome or whole exome bulk sequencing. We extensively validated the tool using both synthetic simulations and single-cell copy number data. Furthermore, we used CNAqc to test CNA calls across 2778 samples from the Pan Cancer Analysis of Whole Genomes (PCAWG), 10 WGS and 48 WES samples from The Cancer Genome Atlas.

CNAqc is a method intended to be integrated in standard WGS and WES analysis, in place of complex consensus-based approaches. Its features can be exploited to clean up data, automatise parameters choice for any caller, prioritise good calls and select what information should be forwarded to downstream analysis.

References

- Greaves, M. and Maley, C.C. (2012) 'Clonal evolution in cancer', Nature, 481(7381), pp. 306–313
- McGranahan, N. and Swanton, C. (2015) 'Biological and therapeutic impact of intratumor heterogeneity in cancer evolution', Cancer cell, 27(1), pp. 15–26.
- McGranahan, N. and Swanton, C. (2017) 'Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future', Cell, 168(4), pp. 613–628.
- The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. Nature 578, 82–93 (2020). https://doi.org/10.1038/s41586-020-1969-6

Multilevel computational approach on transcriptomic data at single cell level in systemic infectious diseases

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The high complexity in patients with systemic infectious diseases, due to bacterial, fungal and viral pathogens, imposed a challenge to design new strategies of clustering patients, exploring molecular variation at different "omics" levels (differentially expressed genes, SNPs, ect.), through complex computation model ad hoc, in order to classify patients with different outcomes.

Here, we propose the application of similarity network models (e.i. PSN) in PBMC samples of 120 healthy subjects exposed to three different pathogens (P. aeruginosa, C. albicans, M. tuberculosis), which would mirror early stages of systemic infectious diseases. This model was built on single cell RNAseq data in PBMC (B cells, T cells, DC, Monocytes and NK) and microbial data, stored in 1M-scBloodNL project (https://eqtlgen.org/sc/datasets/1m-scbloodnl-dataset.html).

These data were used to determine distinct subgroups of PBMCs, belonged to different patients, when these are exposed to specific microbial pathogens, causing small molecular variations in transcriptomic data per single cells. Moreover, it was observed that the monocytic component contributed significantly to the clustering.

This work allowed to apply similarity network model to systemic infectious diseases, defining PBMCs sample clusters with different microbial exposition, featured by transcriptomic signature variations in specific cell types of PBMCs. This experience drives to improve molecular resolution power in systemic infectious diseases, adding other "omics" data in both human host (eQTL, whole exome, blood proteomics, etc.) and pathogen (WGS, AMR and virulence genomic profiling).

References

- Pai S, Bader GD. Patient Similarity Networks for Precision Medicine. J Mol Biol. 2018;430;
- Bhalla et al. Patient similarity network of newly diagnosed multiple myeloma identifies patient subgroups with distinct genetic features and clinical implications. Sci Adv. 2021;7(47):eabg9551.;
- B. Wang,et al., Goldenberg, Similarity network fusion for aggregating data types on a genomic scale. Nat. Methods 11, 333–337 (2014);
- Oelen R, et al. Single-cell RNA-sequencing of peripheral blood mononuclear cells reveals widespread, context-specific gene expression regulation upon pathogenic exposure. Nat Commun. 2022;13(1):3267.

Disentangling the interactions between pathways: a case study on breast cancer

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Background: Current sequencing technologies typically results in long gene lists whose biological interpretation is a major challenge. Pathway analysis is a valuable tool to extrapolate information from these lists, as it reduces the heterogeneity present at gene level and provide insights into the mechanistic functioning of the samples. Pathways are composed by gene products that interact and influence one another to exert a specific cellular function. However, interactions occurs not only within each pathway, but also among them in a phenomenon called pathway cross-talk (PCT). PCT gives explanation on how different cellular mechanisms are mutually influenced. Beside PTC relevance, it is still poorly studied, also due to the lack of dedicated analytic tools. Exploring these interactions is crucial as might underlay pathological phenotypes, or influence the treatment effectiveness, by escaping the drug effect with pathways cross-activation. This study presents an example of PCT analysis using our newly developed tool, Ulisse[1], on TCGA breast cancer (BC) data, involving gene mutations and expressions. Ulisse quantifies the PCT jointly analysing the altered genes, their molecular interactions and their pathway membership, and provides a statistical evaluation through permutations.

BC data (gene mutation and expression) were downloaded from TCGA (TCGAbiolinks[2]). Expression data were analysed to obtain differently expressed genes, while frequency of mutation was calculated from mutation data. Top 300 genes of both analyses were joint in a unique top gene list, that have been provided, together with STRING network, to Ulisse. PCT analysis was performed by using Hallmarks pathways (msigdbr[3]). This workflow was repeated also considering specific tumor subtypes.

We analysed the PCTs affected by genes carrying mutations or altered expression level in BC and all subtypes. We observed PCTs affected in all subtypes, with overall differences in terms of number of cross-talks, and more importantly, subtype-specific PCTs, which could provide valuable insights into the molecular basis of the different features and treatments' outcome among subtypes.

The analysis of PCTs shed light to complex interactions among pathways, which are affected in cancer to inhibit or activate the signalling. This analysis provides actionable targets for further studies in novel therapeutic approaches.

References

1. Chiodi A, Nale V and Mosca E (2022) Ulisse: an R package to go beyond the boundaries of knowledge of molecular pathways [version 1; not peer reviewed]. F1000Research (slides). Doi: 10.7490/f1000research.1118917.1
2. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot T, Malta TM, Pagnotta SM, Castiglioni I, Ceccarelli M, Bontempi G, Noushmehr H (2015). " TCGAbiolinks: An R/Bioconductor package for integrative analysis of TCGA data." Nucleic Acids Research. doi: 10.1093/nar/gkv1507
3. Igor Dolgalev (2021). msigdbr: MSigDB Gene Sets for Multiple Organisms in a Tidy Data Format. R package version 7.4.1. https://CRAN.R-project.org/package=msigdbr

Evaluation of R257C substitution on ACTG2 monomer and filament

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Visceral myopathy is a rare disease caused by smooth muscle weakness in the bowel, bladder, and uterus. It is manifested as feeding intolerance, vomiting, intractable constipation, dramatic bladder dilatation with obstructive uropathy, and uterine atony. The disease is associated with genotype and phenotype variability, so each individual presents a different outcome. Actins are highly conserved proteins, involved in cell motility and in the maintenance of the cytoskeleton. Actin-γ2, smooth muscle (ACTG2) is expressed in enteric tissues. Moreover, heterozygous point mutations in ACTG2 are the primary cause of visceral myopathy. Tens of ACTG2 mutations are implicated in visceral myopathy, while the most common is arginine 257 to cysteine (R257C), which causes a severely pathological phenotype. We performed a molecular dynamics analysis to understand how this mutation affects the stability of the globular protein, as well as the smallest filament unit, a trimer.
The analysis has been performed on AlphaFold model of the globular form (#P63267), in order to investigate all the in vivo conditions, namely bound to ATP or ADP and with phosphate sensor His73 methylated or unmodified; all these conditions have been tested for wild type and mutant ACTG2. Results show that the R257C mutation does not affect the globular form of the protein under any condition considered for the analysis, but the His73 methylation results involved in maintaining the protein's stability.

To better understand the possible effects of this mutation on the filament, we investigate the multimer composed by three monomers, in presence of ADP, homology modelled based on (PDBid: 7jh7). Preliminary analysis shows that R257C mutation has an impact on the intermolecular interactions between monomers. In particular, the number of inter-chain hydrogen bonds is increased in mutant trimer; residue substitution also influences structural flexibility, increasing the mobility of secondary structural elements at chains interface in the near of residue 257. Preliminary results of the effects of R257C substitution indicate a small influence of the substitution on monomer conformation, while a significant impact on filament stability and maintenance, and possible effects on filament conformation.

References

- Hashmi, S. K., Ceron, R. H. & Heuckeroth, R. O. Visceral myopathy: Clinical syndromes, genetics, pathophysiology, and fall of the cytoskeleton. American Journal of Physiology - Gastrointestinal and Liver Physiology vol. 320 G919–G935 Preprint at https://doi.org/10.1152/AJPGI.00066.2021 (2021).
- Kudryashov, D. S. & Reisler, E. ATP and ADP actin states. Biopolymers vol. 99 245–256 Preprint at https://doi.org/10.1002/bip.22155 (2013).
- Hild, G., Bugyi, B. & Nyitrai, M. Conformational dynamics of actin: Effectors and implications for biological function. Cytoskeleton 67, 609–629 (2010).
- Zsolnay, V., Katkar, H. H., Chou E , S. Z., Pollard, T. D. & Voth, G. A. Structural basis for polarized elongation of actin filaments. doi:10.1073/pnas.2011128117/-/DCSupplemental.
- Jepsen, L. & Sept, D. Effects of Nucleotide and End-Dependent Actin Conformations on Polymerization. Biophys J 119, 1800–1810 (2020).

Gut microbiota markers associated with extreme longevity in healthy Sardinian subjects

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Due to its impact on human metabolism and immunology, the gut microbiota (GM) has been proposed as a possible determinant of healthy aging [1]. The present study is aimed at characterizing the gut microbiota (GM) and its functional profile in two groups of Sardinian subjects with a long healthy life expectancy [17 centenarians (CENT) and 29 nonagenarians (NON)] by comparing them to 46 healthy younger controls (CTLs). In addition, the contribution of genetics and environmental factors to the GM phenotype was assessed by comparing a subgroup of 7 centenarian parents (CPAR) with a paired cohort of centenarians' offspring (COFF).

Total DNA was extracted, purified and quantified from each subject's stool sample. Bacterial load was estimate by qPCR and barcoded amplicon libraries were generated using primers targeting the V3 and V4 hypervariable region of the bacterial 16S rRNA gene. Genomic libraries were quantified, normalized, pooled and sequenced on the Illumina MiSeq platform. Operational Taxonomic Unit mapping to the Greengenes database were performed using the Quantitative Insights Into Microbial Ecology (QIIME) platform.

The Verrucomicrobia phylum was identified as the main biomarker in CENT, together with its members Verrucomicrobiaceae, Akkermansia and Akkermansia muciniphila. In NON, the strongest associations concern Actinobacteria phylum, Bifidobacteriaceae and Bifidobacterium, while in CTLs were related to the Bacteroidetes phylum, Bacteroidaceae, Bacteroides and Bacteroides spp. Intestinal microbiota of CPAR and COFF did not differ significantly from each other. Several significant correlations between bacterial taxa and clinical and lifestyle data, especially with Mediterranean diet adherence, were observed.

Our findings seem to define a harmonically balanced intestinal community structure, in which the increase in taxa associated with intestinal health would limit and counteract the action of potentially pathogenic bacterial species in centenarians. The GM of long-lived individuals showed an intrinsic ability to adapt to changing environmental conditions, as confirmed by functional analysis. The GM analysis of centenarians' offspring suggest that genetics and environmental factors act synergistically as a multifactorial cause in the modulation of GM towards a phenotype similar to that of centenarians, although these findings need to be confirmed by larger study cohorts and by prospective studies.

References

1. Ghosh, T. S., Shanahan, F., & O'Toole, P. W. (2022). The gut microbiome as a modulator of healthy ageing. Nature reviews. Gastroenterology & hepatology, 19(9), 565–584. https://doi.org/10.1038/s41575-022-00605-x

Blending biology and bioinformatics

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It is mandatory, but statistical significance is not enough. This brief communication points to share a reflection arising from the experience of recent years in carrying out our studies. I deal with proteomics, in particular with alterations of membrane proteomes or extracellular vesicles following infectious or cancerous pathologies. In recent years, the technological advances concerning the tools applied to the -omics have allowed increasingly in-depth investigations, producing results with thousands of annotations. Further, the sea of statistical probabilities of biological significance swells in complexity by orders of magnitude every time one tries to systematize the results produced by more than one omics. The researchers, bewildered in this sea, rely on bioinformatics software and are confident that candidate biomarkers will emerge, autonomously and almost magically. The experience gained in the last 10 years leads us to share a reflection that we believe is essential in order to be able to face this leap in the complexity of biological and above all biomedical investigation. Software are often based on “predictive” interactions or “old” literature or in the best cases on quantitative data. Not all researchers still have the competence to define (bioinformatics) or choose (biologists) the criteria with which to set the parameters and their stringency. Importantly, we know that often not the most abundant molecules are most interesting or not always the quantitative data, obtained with one type of –omics, correlates with the data of a second –omics, because a standardization of multi-omics analyses is still missing or because there are compensatory mechanisms in the biological system under study. Basically, we are saying that in front of outputs that admits more and more "statistically significant interpretations", the definition of an extremely well-defined and accurate experimental design, and the competence of the researchers must be solidly enough to eliminate from the results as much ambiguity as possible, and finally to recognize the real biomedical significance of the results. We are all part of a system of pressure towards a massive production of data, but what is for increasing exponentially the complexity of produced data, if we are not trained to comprehend the complexity we would like to investigate? And without this acquired competence, how will we be able to create databases and bioinformatics tools that really support the understanding of biological complexity?

Metabolite identification in tandem MS untargeted metabolomics studies

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Untargeted metabolomics with MS techniques allows acquisition of thousands of metabolite signals in a single sample which demands computational techniques and a database for metabolomic identification, despite the existence of different databases, identification remains the bottleneck in metabolomics analysis because of high fragmentation pattern variability in different web-based libraries. We introduce an in-house library that increases the accuracy of fragment matching, and a pipeline that analyzes the complete workflow for metabolomic profiling, specifically addressing the challenges of metabolite identification in LC-MS/MS untargeted metabolomics studies.

The pipeline is implemented in R and is part of the R package “margheRita”. The metabolite identification is implemented in the following functions: “select_library”, which loads the appropriate reference, considering ionization mode and type of chromatographic column; “check_RT” and “check_mass” which quantifies the RT error, m/z ppm error respectively and “check_peaks” quantifies the m/z ppm error and the intensity of their fragments; “metabolite_correlation” quantifies the correlation between metabolites; “visualize_spectra” visualizes two spectra; “metabolite_identification”, a wrapper that executes the full pipeline. The metabolite library was created on 4 different chromatographic columns. The original sample set RP-C18 was obtained by processing urine samples from a kinetic experiment.

Level 1 annotation of features against the in-house reference library includes RT, m/z accurate mass for precursor, and MS/MS fragmentation, along with a series of metabolite descriptors. The annotation process consists of two main steps: firstly, the given features are screened by matching their RT and mass accuracy with the library. Secondly, the MS/MS spectra of features that passed positively on the first screening are compared with the library. Our pipeline also provides two approaches to improve the identification of remaining unknown compounds which are done by assessing, the similarity of MS/MS spectra, and studying the correlation of metabolite levels across samples. We tested the validity of proposed pipeline by several known metabolites and then illustrate the pipeline using urine samples generated for this study.

In conclusion, Annotation with a high-quality spectral in-house library at level 1 enables us to increase the identification of metabolites compared to all available open-access software for efficient use of SWATH datasets for untargeted metabolomic analysis.

References

- Kind T, Tsugawa H, Cajka T, Ma Y, Lai Z, Mehta SS, Wohlgemuth G, Barupal DK, Showalter MR, Arita M, Fiehn O. Identification of small molecules using accurate mass MS/MS search. Mass Spectrom Rev. 2018 Jul;37(4):513-532. doi: 10.1002/mas.21535. Epub 2017 Apr 24. PMID: 28436590; PMCID: PMC8106966.
- Herrera-Lopez S, Hernando MD, García-Calvo E, Fernández-Alba AR, Ulaszewska MM. Simultaneous screening of targeted and non-targeted contaminants using an LC-QTOF-MS system and automated MS/MS library searching. J Mass Spectrom. 2014 Sep;49(9):878-93. doi: 10.1002/jms.3428. PMID: 25230185.
- Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, Kanazawa M, VanderGheynst J, Fiehn O, Arita M. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nat Methods. 2015 Jun;12(6):523-6. doi: 10.1038/nmeth.3393. Epub 2015 May 4. PMID: 25938372; PMCID: PMC4449330.

The pivotal role of bioinformatic analysis in the genomic characterization of two non-K1 Escherichia coli isolates responsible of meningitis to update molecular assays for meningitis diagnosis

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Typically, Escherichia coli strains causing meningitis carry K1 capsule. Currently available methods, employed to identify the principal etiological agents of meningitis, consist of a multiplex real time PCR targeting only K1 Escherichia coli and other microorganism responsible of meningitis. However, two Escherichia coli strains, determining adult and fatal neonatal meningitis, were isolated from cerebrospinal fluids bacterial culture, even if the molecular method failed to identify them, leading to a false negative result. To characterize these Escherichia coli strains and to understand their capability to cause meningitis, whole genome sequencing (WGS) was performed.
Identification of isolates was performed with MALDI-TOF (BioMérieux, Italy). WGS was performed through Illumina MiSeq platform. Multilocus Sequence Type (MLST) was obtained through Enterobase. In silico analysis to identify antimicrobial resistance genes, plasmids and for serotyping were performed through bioinformatic tools of Center for Genomic Epidemiology of Technical University of Denmark. Virulent Factor Database (VFDB) using VFanalyser tool was implemented for virulence factors detection. The presence in the genome of each gene, detected by bioinformatic tools, was further assessed mapping isolates raw reads to the exact gene sequence of the concerned gene.

In silico WGS analysis showed that these Escherichia coli strains belonged to ST131 (phylogenetic group B2, O25:H4 serotype) and ST69 (phylogenetic group D, O15:H18 serotype). Genomic characterization confirmed the absence of K1 capsule and any other capsule serotypes and led to the identification of ibe genes in both Escherichia coli isolates, which presumably could be responsible for invasion of brain endothelial cells. These Escherichia coli strains were assigned the virulence score of 6 and 4 respectively, according to the presence of 11 virulence factors detected with genomic analysis.
In vitro antimicrobial susceptibility tests were consistent with antimicrobial resistance genes detected in both genomes. Escherichia coli isolate 1 was a multi-susceptible strain while the other was a multi-drug resistant one. Escherichia coli isolate 2 harbored a plasmid carrying blaTEM gene for β-lactams resistance.

Our findings highlight the crucial role of genomic characterization of emerging non K1 Escherichia coli strains through bioinformatic tools to update new molecular methods for a more reliable meningitis diagnosis.

References

- Ouchenir L, Renaud C, Khan S, Bitnun A, Boisvert AA, McDonals J, Bowes J, Brophy J, BartonM, Ting J, Roberts A, Hawkes M, Robinson JL. 2017. The epidemiology, management, and outcomes of bacterial meningitis in infants. Pediatrics 140: e20170476.
- Bodilsen J, Brouwer MC, Kjærgaard N, Sirks MJ, van der Ende A, Nielsen H, van de Beek D, DASGIB study group. 2018. Community-acquired meningitis in adults caused by Escherichia coli in Denmark and the Netherlands. J Infect 77:25e9
- Moussiegt A, Birgy A, Cointe A, Duval X, Bidet P, Bonacorsi S. 2022. Escherichia coli community-acquired meningits in adults: a case series of 29 patients in France. Clin Microbiol Infect https://doi.org/10.1016/j.cmi.2022.04.020
- Plasmid finder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder-2.0/) 5. Serotype Finder 2.0 (https://cge.food.dtu.dk/services/SerotypeFinder/) 6. Virulent Factor Database (VFDB) through VFanalyser tool (http://www.mgc.ac.cn/cgi bin/VFs/v5/main.cgi)
- ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/)
- Zhou Z, Alikhan NF, Mohamed K, the Agama Study Group, Achtman M (2020) The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny and Escherichia core genomic diversity. Genome Res. 30:138-152. 9. Geneious software (v. 11.1.5) (Biomatters Ltd., Auckland, New Zealand).

Identification of trans factors regulating Tau exon 6 alternative splicing

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The Microtubule-Associated Protein Tau (MAPT) gene encodes for a microtubule binding protein fundamental for microtubule stabilization [1]. Altered expression of Tau isoforms is associated to a group of disorders collectively referred to as tauopathies, which include Alzheimer's disease and Pick Disease [2]. The human MAPT gene is composed of 16 exons and, while some of them are constitutively spliced into the final transcript, exon 2, 3, 4a, 6, 8 and 10 are subjected to alternative splicing (AS) [3]. Over the years, widely diverse Tau isoforms generated from different transcripts have been reported and characterized [1], and while the different function of Tau protein isoforms is currently studied and characterized, the splicing regulation of the transcripts is still poorly understood. The splicing regulation of Tau exon 6, known to be expressed in a tissue-dependent manner contributing to a different balance of functional Tau, has not been previously investigated. Therefore, the aim of this work was to develop a molecular tool employable to study Tau exon 6 (E6) splicing regulation by different RNA binding proteins (RBPs).

To this aim, the web-based RBP-binding prediction tool RBPmap [4] was used to scan the genomic region flanking Tau E6 to identify potential binding sites for RBPs of interest (PTB, nPTB and RBM20). Then, after selecting the exon 6-flanking genomic region, a molecular cloning approach was used to develop an exon 6 minigene clone containing the putative intron regulatory sequences into the RHCglo vector [5]. The minigene was co-transfected with RBP expressing vectors into Hek293T cell. RNA was purified by transfected cells and analyzed by RT-PCR.

In this work we report the successful employment of a bioinformatic pre-screening to identify potential regions of interest in the splicing regulation of a selected exon. Furthermore, we were able to produce a minigene vector suitable to study the Tau E6 splicing mechanisms. We also report preliminary results suggesting the potential regulation of Tau E6 splicing by a tissue-specific alternative splicing regulatory factor RBM20. Further experiments will clarify the interplay of alternative splicing factors in the molecular mechanisms which regulate Tau E6 splicing.

References

- Ruiz-Gabarre, D.; Carnero-Espejo, A.; Ávila, J.; García-Escudero, V. What's in a Gene? The Outstanding Diversity of MAPT. Cells 2022, 11, 25, doi:https://doi.org/10.3390/cells11050840.
- Zhang, Y.; Wu, K.-M.; Yang, L.; Dong, Q.; Yu, J.-T. Tauopathies: New Perspectives and Challenges. Mol. Neurodegener. 2022, 17, 28, doi:10.1186/s13024-022-00533-z.
- Andreadis, A. Tau Gene Alternative Splicing: Expression Patterns, Regulation and Modulation of Function in Normal Brain and Neurodegenerative Diseases. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 2005, 1739, 91–103, doi:10.1016/j.bbadis.2004.08.010. 4. Paz, I.; Kosti, I.; Ares, M.; Cline, M.; Mandel-Gutfreund, Y. RBPmap: A Web Server for Mapping Binding Sites of RNA-Binding Proteins. Nucleic Acids Res. 2014, 42, W361–W367, doi:10.1093/nar/gku406.
- Singh, G.; Cooper, T.A. Minigene Reporter for Identification and Analysis of Cis Elements and Trans Factors Affecting Pre-MRNA Splicing. BioTechniques 2006, 41, 177–181, doi:10.2144/000112208.

Comparison of tools for Intercellular Communication Inference from Single-Cell Transcriptomics Data

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The study of cell–cell communication (CCC) is one of the relevant opportunities provided by single cell (SC) transcriptomics. Various tools have been developed to address this task. The majority of tools focuses on gene level analysis, while few of them provide intercellular communication information at cell cluster level. The recently developed method (Ulisse) considers the quantification of interactions at cell-cluster level. It calculates inter-cluster communication scores on the basis of Ligand-Receptor interactions, cluster-level "marker" genes, and assesses the statistical significance by means of an empirical null model. Here, we compare Ulisse with existing tools (SingleCellSingleR, Icellnet, and CellChat) on normal mammary gland and breast cancer SC transcriptomics datasets.

SC datasets: the four organoids of breast healthy tissue (GSE113197) and three breast cancer samples, choosen by cell number and variety of cell types (CID4290A, CID44991, CID4515; from GSE176078). SC data were analysed by using Seurat. Correlation analysis was performed using Spearman's correlation. Topology analysis was based Jaccard index and a dissimilarity score based on Jensen Shannon Divergence. Robustness analysis was done calculating the performance (AUC) in recovering the same results with subsampled input data.

We run the four tools on seven SC transcriptomics datasets. In each dataset, we obtained, for each tool, a list of communication scores between all-pairs of cell clusters. We compared these results by correlation analysis, topological analysis of the Intercluster Communication Networks (ICNs) and performance against input data subsampling. We found significant variability between tools, especially between the ICNs.

The discrepancies we observed can be brought up to the use of different ligand-receptor interaction data and score calculation method. In general, the comparison of CCC tools is complicated by the absence of verified ground truths. Overall, Ulisse showed good performances and provides a statistical assessment of the results.

References

- A. Almet, Zixuan Cang, Suoqin Jin, Qing Nie, The landscape of cell–cell communication through single-cell transcriptomics, Current Opinion in Systems Biology, Volume 26, 2021, Armingol, E., Officer, A., Harismendy, O. et al. Deciphering cell–cell interactions and communication from gene expression. Nat Rev Genet 22, 71–88 (2021).

Genome-wide association studies of advanced cancer patients identified loci associated with opioid-induced nausea-vomiting

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Advanced cancer patients usually receive opioids to treat cancer pain; unfortunately, 10-20% do not benefit from treatment and even higher percentages experience side effects such as nausea and vomiting. Previous studies suggested a possible role of genetics in determining individual variability in the response to opioids. The aim of this genome-wide association study is to identify new genetic markers of opioid toxicity.

The study included 1,038 European cancer patients receiving morphine, oxycodone, buprenorphine or fentanyl. The patients were included in one cross-sectional and two longitudinal studies. Nausea and vomiting was measured by the European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC-QLQ-C30). A composite nausea-vomiting score (NVS), ranging from 0 to 100, was calculated. Patients were genotyped using Axiom PMRA arrays. Genome-wide linear regressions between NVS and the genotypes of 432,087 variants were performed using PLINK software.

NVS significantly associated ($P<5.0x10-8$) with the genotype of 12 variants (six, five, and one on chromosome 2, 6 and 15, respectively). The top-significant variants at each locus were rs6723108 (beta=7.26, $P=7.47x10-11$), rs2596503 (beta=10.0, $P=2.96 x10-11$), and rs1129138 (beta=-5.81, $P=1.65 x10-9$), respectively. The gene nearest to rs6723108 is TMEM163, encoding a transmembrane protein transporting zinc ions, whereas variants on chromosomes 6 mapped in the HLA locus.

These preliminary results indicate that opioid induced nausea and vomiting are regulated by germline variants. However, further studies are needed to validate these results and to identify the molecular mechanisms underlying the observed associations.

References

- Caraceni A, Hanks G, Kaasa S, Bennett MI, Brunelli C, Cherny N, et al. Use of opioid analgesics in the treatment of cancer pain: Evidence-based recommendations from the EAPC. Lancet Oncol 2012;13:e58-68.
- Colombo, F., Pintarelli, G., Galvan, A. et al. Identification of genetic polymorphisms modulating nausea and vomiting in two series of opioid-treated cancer patients. Sci Rep 10, 542 (2020). https://doi.org/10.1038/s41598-019-57358-y
- Galvan A, Skorpen F, Klepstad P, Knudsen AK, Fladvad T, Falvella FS, et al. Multiple loci modulate opioid therapy response for cancer pain. Clin Cancer Res 2011;17:4581-7
- Oosten AW, Oldenmenger WH, Mathijssen RH, van der Rijt CC. A systematic review of prospective studies reporting adverse events of commonly used opioids for cancer-related pain: A call for the use of standardized outcome measures. J Pain 2015;16:935-46.

HPV16: first interactive map between human proteins and the viral genome

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Papillomavirus (HPV) is characterized by infecting cells in active proliferation such as the epithelial cells of the epidermis and mucous membranes. The infection affects both women and men, especially at a young age. HPV is present in 99.7% of cervical cancer in women and a number of cancers with variable incidence in the population; we include among these pathologies, anal cancer, some rare forms and oropharyngeal. Among the 200 known HPV genotypes, 16 and 18 are considered to be at high oncogenic risk. The aim of this project is to identify all human proteins that interact with the HPV 16 genome in order to identify new pharmacological strategies.

Hybrid oligos were designed using Primer 3 software for the entire HPV 16 genome. Plasmid phpv-16 in the bacterium E.coli (ATCC, #45113) was amplified, extracted and used as a template in the first phase of the Polymerase Chain Reaction (PCR) pull down. The first PCR amplifies the region of interest with the use of hybrid Primers that carry a specific region and a second unique region not present in the genome. The 500 bp amplifiers, thus obtained, were used as templates in the second PCR, to obtain fragments with biotinyzed ends in 5'. In the meantime, the nuclear extraction of SiHa cells (Elabscience EP-CL-0210) has been conducted, containing about 3 integrated copies of HPV16 for cell. Subsequently, DNA pull-down was carried out: the biotinylated dsDNA fragment interacted with the Streptavidin Sepharose beads and the nuclear extract was added to this complex. The final product was analysed using Liquid Chromatography Mass Spectrometry-Mass Spectrometry.

About 4350 proteins were identified by mass spectrometry analysis and 310 of these were unique. Gene ontology (GO) was carried out by analyzing biological processes, molecular functions and cellular components in which identified interactors participate. Many of these are involved in DNA replication, transcription and translation processes.

This interactive map could provide important advantages both in understanding the mechanism of integration of the virus in the host cell, as yet unknown, and to discover new molecular targets for specific therapies for these tumors.

References

- Olusola P, Banerjee HN, Philley JV, Dasgupta S. Human Papilloma Virus-Associated Cervical Cancer and Health Disparities. Cells. 2019 Jun 21;8(6):622. doi: 10.3390/cells8060622. PMID: 31234354; PMCID: PMC6628030.

In silico design and evaluation of exon-skipping strategies induced by antisense oligonucleotides for therapeutic intervention in cancer

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Many cancer driver genes still undruggable at the protein level may prove druggable at mRNA level, as by using the potential approach of targeted exon skipping. To this end, antisense oligonucleotides (ASOs) can be used to bind to complementary sequences, thus modulating alternative splicing to produce desired protein products.

We developed a pipeline, by using the Python programming language, to identify exons amenable to be skipped in a gene of interest. For each annotated transcript of the given input gene, the computational pipeline classifies as in-frame or out-of-frame each shortened transcript product resulting from in-silico skipping of its exons. All out-of-frame transcript products are then subjected to prediction of their degradation through Non-sense Mediated Decay (NMD). Moreover, the pipeline designs ad hoc ASOs that could promote the skipping of a specific exon of interest by targeting the splice-junctions or internal splice regulatory regions. A further procedure evaluates identified ASOs based on reference chemo-physical parameters to select best candidate sequences. Furthermore, analysis of cancer mutation profiles based on public data is used to rank most mutated exons and transcripts.

We run the pipeline on a list of 72 genes selected as the top 10% mutated genes in cancer from public databases. Here, we observe a mean of nine protein-coding transcripts annotated per gene, with each transcript having at least one possibly skippable exon. Importantly, a mean of over 26 exons were potentially amenable to be skipped for each gene across all its annotated transcripts. Out of the 72 genes, 94% (68/72) are computationally predicted to be able to provide both in-frame and out-of-frame shortened transcripts, thus allowing potential therapeutic exon-skipping design towards both outcomes: inactivation or functional rescue.

In this work, we present a computational pipeline implementing state-of-the-art rules for cancer gene classification as oncogenes or tumor-suppressors, and for designing ASOs to induce targeted exon skipping. In particular, this pipeline may serve useful towards therapeutic exon-skipping strategies aimed at producing desired protein variants for selected cancer genes, such as inactive variants of oncogenes or tumor suppressors at least partially rescued in function.

References

- Moulton, J. D., & Yan, Y. L. (2008). Using Morpholinos to control gene expression. Current protocols in molecular biology, Chapter 26(1), 2681–26.8. https://doi.org/10.1002/0471142727.mb2608s83
- Moulton, Jon. (2016). Guide for Morpholino Users: Toward Therapeutics. Journal of Drug Discovery, Development and Delivery. 3. 1023.
- Yokota, T., Duddy, W., & Partridge, T. (2007). Optimizing exon skipping therapies for DMD. Acta myologica : myopathies and cardiomyopathies : official journal of the Mediterranean Society of Myology, 26(3), 179–184.
- Cale JM, Greer K, Fletcher S, Wilton SD. Proof-of-Concept: Antisense Oligonucleotide Mediated Skipping of Fibrillin-1 Exon 52. Int J Mol Sci. 2021 Mar 27;22(7):3479. doi: 10.3390/ijms22073479. PMID: 33801742; PMCID: PMC8037683.
- Leoni G, Le Pera L, Ferrè F, Raimondo D, Tramontano A. Coding potential of the products of alternative splicing in human. 2011 Genome Biol. 12(1):R9.
- Le Pera L, Mazzapioda M, Tramontano A. 3USS: a web server for detecting alternative 3'UTRs from RNA-seq experiments. 2015 Bioinformatics. 31(11):1845-7.

Dynamics of Gut Microbiota and Clinical Variables after Ketogenic and Mediterranean Diets in Drug-Naïve Patients with Type 2 Diabetes Mellitus and Obesity

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Type 2 diabetes mellitus (T2DM) is a progressive chronic metabolic disease that has increasingly spread worldwide, enhancing the mortality rate, particularly from cardiovascular diseases. Lifestyle improvement through diet and physical activity is, together with drug treatment, the cornerstone of T2DM management. The Mediterranean diet (MD is usually recommended [1,3], and, recently, scientific societies have promoted a very low-calorie ketogenic diet (VLCKD), a multiphasic protocol that limits carbohydrates and then gradually reintroduces them, with a favorable outcome on body weight and metabolic parameters [4,5]. The gut microbiota (GM) modifications have been linked to overweight/obesity and metabolic alterations typical of T2DM. Diet is known to affect GM largely, but only a few studies have investigated the effects of VLCKD on GM [6,7].

We have compared the clinical parameters, the quality of life and the GM of eleven patients with recently diagnosed T2DM and overweight or obesity, randomly assigned to two groups of six and five patients who followed the VLCKD (KETO) or hypocaloric MD (MEDI), respectively; data were recorded at baseline (T0) and after two (T2) and three months (T3) of nutritional intervention. The GM analysis was performed through Next Generation Sequencing of the V3 and V4 hypervariable region of the 16S rRNA gene on the MiSeq Illumina platform.

The results showed that VLCKD had more significant beneficial effects than MD on anthropometric parameters, while biochemical improvements did not statistically differ. As for the GM, in the KETO group, a significant increase in beneficial microbial taxa such as Verrucomicrobiota phylum with its members Verrucomicrobiae, Verrucomicrobiales, Akkermansiaceae and Akkermansia, Christensenellaceae, Eubacterium spp., and a reduction in microbial taxa previously associated with obesity or other diseases was observed both at T2 and T3. In the MEDI group, variations were limited to a significant increase in Actinobacteroidota at T2 and T3 and Firmicutes phylum at T3. Moreover, alterations linked to some metabolic pathways were found exclusively in the KETO group.

In conclusion, both dietary approaches allowed patients to improve their state of health, but VLCKD has shown better results on body composition as well as on GM profile.

References

- Martínez-González, M.A.; Gea, A.; Ruiz-Canela, M. The Mediterranean Diet and Cardiovascular Health. Circ. Res. 2019, 124, 779–798.
- Mazzocchi, A.; Leone, L.; Agostoni, C.; Pali-Schöll, I. The Secrets of the Mediterranean Diet. Does [Only] Olive Oil Matter? Nutrients 2019, 11, 2941.
- Martín-Peláez, S.; Fito, M.; Castaner, O. Mediterranean Diet Effects on Type 2 Diabetes Prevention, Disease Progression, and Related Mechanisms. A Review. Nutrients 2020, 12, 2236.
- Paoli, A.; Rubini, A.; Volek, J.S.; Grimaldi, K.A. Beyond weight loss: A review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. Eur. J. Clin. Nutr. 2013, 67, 789–796.
- Ludwig, D.S. The Ketogenic Diet: Evidence for Optimism but High-Quality Research Needed. J. Nutr. 2020, 150, 1354–1359.
- Rawat, K.; Singh, N.; Kumari, P.; Saha, L. A review on preventive role of ketogenic diet (KD) in CNS disorders from the gut microbiota perspective. Rev. Neurosci. 2021, 32, 143– 157.
- Cabrera-Mulero, A.; Tinahones, A.; Bandera, B.; Moreno-Indias, I.; Macías-González, M.; Tinahones, F.J. Keto microbiota: A powerful contributor to host disease recovery. Rev. Endocr. Metab. Disord. 2019, 20, 415–425.

Finding a Needle in a Haystack: Impact of Shotgun Metagenomics Sequencing Depth on Microbial Identification and Antimicrobial Resistance Genes Detection

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Shotgun metagenomics sequencing experiments are finding a wide range of applications [1], nonetheless there are still limited guidelines regarding the depth of sequencing needed to acquire meaningful information, especially regarding antimicrobial resistance genes (ARGs) identification. In this work, we analyzed the impact of sequencing depth on microbial identification and ARGs detection in order to give helpful insights into designing shotgun metagenomics experiments to return the maximum useful information with the minimum cost possible.

We sequenced 4 human plaque samples and 1 Microbial Community Standard, and generated in silico a total of 40 sequences datasets simulating a variety of sequencing depth. All datasets underwent quality controls including adapter and quality trimming, and host DNA contamination removal (KneadData tool). Once a good-quality microbial sequences dataset was obtained, a quantitative profiling of microbial communities composition was carried out using MetaPhlAn3 v3.1.0 tool [2] and ARGs presence was assessed by aligning sequence reads to MEGARes 2.0 database [3].

When investigating the impact of sequencing depth on quantitative taxonomic profiling in the Microbial Community Standard datasets, we found some discrepancies in the identified microbial species and their abundances when compared to the reported ones. Such differences are however consistent across different sequencing depth, suggesting that they are instead linked to limits of the taxonomic profiling methods. Overall, results showed that sequencing depth has a great impact on metagenomic samples both at qualitative (i.e. presence/absence) and quantitative level in terms of loss of information, especially in datasets having <35 millions of reads. The presence of ARGs was also assessed: overall, a total of 133 ARGs were identified and 23% of them did not consistently result as present or absent across different sequencing depth datasets of the same sample. In particular, we observed that the information loss increased at diminishing sequencing depth, with more than half of such genes lost at <20 million of reads.

In conclusion, samples with reduced sequencing depths decrease resolution for microbiome qualitative and quantitative profiling and hinder ARGs identification. This study highlights the importance of carefully considering such aspects in designing shotgun metagenomics experiments to maximize microbiome analyses.

References

- Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. Nat Biotechnol. 2017 Sep;35(9):833–44.
- Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. Turnbaugh P, Franco E, Brown CT, editors. eLife. 2021 May 4;10:e65088.
- Doster E, Lakin SM, Dean CJ, Wolfe C, Young JG, Boucher C, et al. MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Research. 2020 Jan 8;48(D1):D561–9.

Long-term immunogenicity of oral poliomyelitis vaccine (OPV) in a polio-free country

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The extensive use of oral and inactivated poliovirus (PV) vaccines has driven progress toward the global eradication of wild PV2 and PV3 and the elimination of PV1 in most countries, including Italy. Although the persistence of circulating neutralizing antibodies among the vaccinated is unclear, it is estimated that >99% of the population vaccinated according to the recommended protocol should be protected for at least 18 years.

This study evaluated the seroprevalence of anti-PV neutralizing antibodies and the long-term immunogenicity of the oral poliovirus vaccine (OPV) in a sample of medical students and residents of the University of Bari who attended the Hygiene Department for a biological risk assessment between April 2014 and October 2020.

1,408 students and residents had received four doses of OPV and were included in this study. The prevalence of protected vaccinated individuals was >90% for PV1, PV2, and PV3. Specifically, > 99% of the study group was protected against PV1, > 98% against PV2, and almost 93% against PV3. None of our analyses indicated that sex affected the seropositivity rate or the immune response to OPV. Protective antibodies against all three viruses persisted for at least up to 18 years after administration of the last OPV dose, with PV1 and PV2 antibodies detected in > 95% of the participants > 30 years after the last OPV dose. In summary, the time between the last vaccination against polio and the antibody titer evaluation is a determinant of the levels of persisting neutralizing antibodies.

The childhood series of four doses of OPV guarantees a long duration of protection, despite the elimination of the virus and therefore the absence of a natural booster. However, until PV1 is completely eradicated, maximum vigilance by public health institutions must be maintained. Future studies should focus on the long-term immunogenicity of mixed vaccination schedules and, above all, on the current four-dose schedule for IPV (plus an additional dose in adolescence), to evaluate any critical issues that may lead to a risk situation in the event of reintroduction of the wild virus.

References

- WHO. Poliomyelitis. Key facts. Available from: https://www.who.int/news-room/fact-sheets/detail/poliomyelitis. CDC. Pink book. Epidemiology and Prevention of Vaccine-Preventable Diseases. Poliomyelitis. Available from: https://www.cdc.gov/vaccines/pubs/pinkbook/polio.html#vaccines. WHO. Biologicals. Oral polio vaccine (OPV). Available from: https://www.who.int/biologicals/areas/vaccines/polio/opv/en/. CDC. Vaccines and Preventable Diseases. Vaccine-derived Poliovirus. Available from: https://www.cdc.gov/vaccines/vpd/polio/hcp/vaccine-derived-poliovirus-faq.html. CDC. Vaccines and Preventable Diseases. Polio Vaccine Effectiveness and Duration of Protection. Available from: https://www.cdc.gov/vaccines/vpd/polio/hcp/effectiveness-duration-protection.html. CDC. Who We Are: CDC and the Global Polio Eradication Initiative. Available from: https://www.cdc.gov/polio/who/index.htm. WHO European Region. Certification of the Region's polio-free status in 2002. Available from: https://www.euro.who.int/en/health-topics/communicable-diseases/poliomyelitis/activities/certification-and-maintenance-of-polio-free-status-in-the-european-region/european-regional-commission-for-the-certification-of-poliomyelitis-eradication/certification-of-the-regions-polio-free-status-in-2002. WHO. Persistence of protective antibodies following immunization with OPV and IPV. Available from: https://www.who.int/immunization/polio_grad_duration_protection.pdf.
- Bandyopadhyay AS, Gast C, Rivera L, et al. Safety and immunogenicity of inactivated poliovirus vaccine schedules for the post-eradication era: a randomised open-label, multicentre, phase 3, non-inferiority trial. Lancet Infect Dis 2020. Published Online October 23, 2020.
- Crovari P. Public Health History Corner History of polio vaccination in Italy. IJPH - Year 8, Volume 7, Number 3, 2010
- Baldo V, Baldovin T, Cocchio S, et al. Seroepidemiology of polioviruses among university students in northern Italy. Clin Vaccine Immunol. 2012;19(8):1292-1295.
- Germinario C, Gallone MS, Tafuri S. Migrant health: the Apulian model. Epidemiol Prev. 2015 Jul-Aug;39(4 Suppl 1):76-80.
- Fine PE, Oblapenko G, Sutter RW. Polio control after certification: major issues outstanding. Bull World Health Organ. 2004;82:47–52. Epub 2004 Feb 26.

Host defence amphibian peptide and its potential as antiviral agent

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Nowadays the unmet need for effective drugs against microbial pathogens and emerging infections is obvious. The World Health Organization (WHO) ruled as a priority the research of new agents able to fight superbugs that are rising. Not only bacteria, parasites, and fungi are acquiring new escape mechanisms, also viruses adopt new strategies to survive. Therefore, the characterization and the development of new antiviral therapies are mandatory. Antimicrobial peptides (AMPs) could represent an important source of new anti-infective agents [1]. They represent the most ancient and fast-acting elements of the host innate defence system against infectious agents. Concerning the fact that several AMPs were full characterized for their antibacterial activities, we investigated the potential of the AMP AR-23 derived from Rana tagoi. The amphibian peptide belongs to one of the largest families and is among the smallest-sized AMP (23 amino acids).

Peptide has been synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. The cytotoxic activity was determined via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on VERO cells. The antiviral activity was evaluated against different members of Herpesviridae (Herpes simplex virus type 1, HSV-1), Paramyxoviridae (measles virus, MeV; Human parainfluenza virus type 2, HPIV-2), Coronaviridae (Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; Human coronavirus 229E, HCoV-229E) and Picornaviridae (Poliovirus, PV-1) families, by using plaque assays, molecular test and Transmission electron microscopy (TEM) analysis. In addition, molecular docking was performed.

Preincubation of peptide with viruses has determined a significant antiviral activity, demonstrating that it could disrupt the viral envelope, also confirmed by TEM. The peptide acts on the extracellular phases of the viral lifecycle, probably by blocking the viral attachment and entry phases. Therefore, our data showed that the peptide analyzed inhibit the infection of coronaviruses in a dose-dependent manner by binding the RBD of the Spike protein, as suggested by molecular docking and validated by cell-based studies. These results show possible novel applications of amphibian skin peptides in the field of antivirals. Further studies will focus on their specific mechanism of action to clarify the viral target on which the peptide act.

References

1. Zannella C. et al., Broad-Spectrum Antiviral Activity of the Amphibian Antimicrobial Peptide Temporin L and Its Analogs. Int J Mol Sci. 2022 Feb; 23(4): 2060.

Novel amino-pyrazole analogues interfering with cell migration

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5-pyrazolyl urea derivatives represent attractive molecules endowed with interesting pharmaceutical properties [1]. In particular, GeGe-3 (Figure 1) showed promising antiangiogenetic activity interfering with MAPK and PI3K signalling pathways and inhibiting proliferation and migration in HUVEC cells [2]. Furthermore, proteomic studies indicated Calreticulin as potential target for this derivative [3]. To further extend the structure-activity relationships of GeGe-3, a novel series of amino pyrazoles was designed and synthesized. The adopted synthetic strategy led to the
formation of new amino-pyrazoles 2 and their carbamate analogues 3 (Figure 1).

All the new derivatives were preliminary tested in MTT assay to evaluate their antiproliferative activity on a panel of tumour and normal cell lines. A Western Blot screening was carried out to assess the compounds' ability to interfere with AKT and ERK1/2 phosphorylation. Moreover, cell proliferation and cell migration tests highlighted interesting antiangiogenetic properties for the prepared compounds. Interestingly, selectedcompounds, proved to be more effective than GeGe-3, designated as reference compound. The synthetic procedures, the structural elucidation as well as the biological properties of the prepared pyrazoles will be discussed in the presentation.

References

1. C. Brullo, F. Rapetti, O. Bruno, Molecules 2020, 25, 3457.
2. E. Meta, C. Brullo, A. Sidibe, B.A. Imhof, O. Bruno, Eur. J. Med. Chem. 2017, 133, 24.
3. E. Moretta, R. Belvedere, A. Petrella, A. Spallarossa, F. Rapetti, O. Bruno, C. Brullo, M.C. Monti, Bioorg. Chem. 2021, 115, 105168.

Structure-Based Virtual Screening (SBVS) in flexible systems: the case of SIRT-2

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Sirtuin-2 is a (NAD+)-dependent enzyme with deacetylase activity [1]. Dysregulation in Sirt-2 activity was shown to be related to a plethora of diseases, making this protein a target of pharmacological interest. In particular, Sirt-2 inhibitors have shown therapeutic potential for the treatment of neurodegenerative disorders, cancer and metabolic diseases [2]. Sirt-2 flexibility is reflected by the abundancy of conformationally diverse 3D structures deposited in the Protein Data bank (PDB) [3], and represents a challenge for in-silico drug discovery, particularly for structure-based virtual screening (SBVS) techniques.

In the present study, a workflow for the evaluation and selection of structures for SBVS was developed, exploring single- and multiple- conformation approaches. The presented procedure comprised the selection of suitable structures through manual inspection and redocking, and the evaluation of their ability to select active compounds within a set of decoys. The latter was evaluated by performing preparatory virtual screenings (VS) with a benchmarking database on the selected structures, and by measuring the corresponding Receiver Operating Characteristic AUC (ROC-AUC). The best result was chosen to screen a central nervous system (CNS) focused library of more than 22000 compounds by ChemDiv [4]. Post-processing involved consensus among two scoring functions and the visual inspection of the resulting poses. 5 compounds were purchased and tested in vitro.

The perspective screenings individuated the 5Y5N crystal structure as the best conformation for VS, according to two distinct scoring functions. None of the multi-conformation screenings outperformed the best single conformation one, and therefore the latter was selected for the prospective VS on the ChemDiv library. In vitro tests of the selected candidates highlighted a weak activity of 3 compounds out of 5.

Despite the limited activity of the hit compounds, a previously unreported scaffold was individuated, which may be of interest for further optimization. The study provided a useful workflow for structure selection in VS.

References

1. Lee, S., Chen, Z. & Zhang, G. Catching Sirtuin-2 Intermediates One Structure at the Time. Cell Chemical Biology vol. 24 248–249 (2017).
2. Wang, Y., Yang, J., Hong, T., Chen, X. & Cui, L. SIRT2: Controversy and multiple roles in disease and physiology. Ageing Research Reviews 55, 100961 (2019).
3. RCSB PDB: Homepage. https://www.rcsb.org/.
4. CNS BBB Library. https://www.chemdiv.com/catalog/focused-and-targeted-libraries/cns-bbb-library/

Glicopro, a multi-modulative ocular formulation based on standardized and sterile snail mucus extract

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This study focused on the evaluation of mucoadhesive, regenerative and anti-microbial properties of a novel lubricating multimolecular ophthalmic solution (GlicoPro®) obtained from snail mucus[1] and its potential anti-inflammatory and analgesic role in the management of eye diseases[2], in comparison with sodium hyaluronate and trehalose.

GlicoPro, sodium hyaluronate and trehalose (Thealoz duo) bio-adhesivity was assessed using a lectin-based assay, and its regenerative properties were studied using human corneal epithelial cell line. In vitro Dry Eye Sindrome (DED) was induced in human corneal tissues; the histology and mRNA expression of selected genes of inflammatory and corneal damage biomarkers were analyzed in DED tissues treated with GlicoPro. Anti-viral properties were tested on in vitro experimental models of Herpes simplex 1 virus (HSV-1) infections (0.01 PFU/cell) on Vero cell line (ATCC-CCL81).

Higher rates of bio-adhesivity were observed in corneal cells treated with GlicoPro than with the other treatments. GlicoPro improved in vitro corneal wound healing during in vitro scratch test. Histo-morphological analysis revealed restoration of cellular organization of the corneal epithelium, microvilli, and mucin network in DED corneal tissues treated with GlicoPro. A significant reduction in inflammatory and ocular damage biomarkers was observed. High-performance liquid chromatography-mass spectrometry analysis identified an endogenous opioid, opiorphin[3], in the peptide fraction of GlicoPro. After 2 hours fromHSV-1 infection of Vero cells, the treatment with GlicoPro was able to reduce 3 log the HSV-1 titer, similar to the effect obtained with acyclovir 10-5 M. The anti-viral effect is dose-dependent, and it is already evident 24 hours after HSV-1 infection.

GlicoPro lead to both regeneration and bio-adhesivity in corneal cells[4]; moreover, in view of its analgesic[5], anti-inflammatory and anti-viral properties, this novel ophthalmic lubricating solution may be an innovative approach for the management of eye.

References

1. Gentili V, Bortolotti D, Benedusi M, Alogna A, Fantinati A, Guiotto A, Turrin G, Cervellati C, Trapella C, Rizzo R, Valacchi G. HelixComplex snail mucus as a potential technology against O3 induced skin damage. PLoS One. 2020 Feb 21;15(2):e0229613. doi: 10.1371/journal.pone.0229613
2. Giannaccare G, Ghelardini C, Mancini A, Scorcia V, Di Cesare Mannelli L. New Perspectives in the Pathophysiology and Treatment of Pain in Patients with Dry Eye Disease. J Clin Med. 2021 Dec 25;11(1):108. doi: 10.3390/jcm11010108.
3. Rougeot, C.; Messaoudi, M. Identification of human opiorphin, a natural antinociceptive modulator of opioid dependent pathways. Med. Sci. 2007
4. Mencucci R, Strazzabosco G, Cristofori V, Alogna A, Bortolotti D, Gafà R, Cennamo M, Favuzza E, Trapella C, Gentili V, Rizzo R. GlicoPro, Novel Standardized and Sterile Snail Mucus Extract for Multi-Modulative Ocular Formulations: New Perspective in Dry Eye Disease Management. Pharmaceutics. 2021 Dec 13;13(12):2139. doi: 10.3390/pharmaceutics13122139.
5. Ozdogan S, Sonmez C, Yolcu D, Gungormus M. Tear Opiorphin Levels in Ocular Pain Caused by Corneal Foreign Body. Cornea. 2020 Nov;39(11):1377-1380

Using human induced pluripotent stem cells technology for the study of neuronal alterations and vulnerability to stress in patients with Treatment-Resistant Depression

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Major depressive disorder (MDD) is a complex and heterogeneous neuropsychiatric disease, characterized by different defective neurotransmitter systems, including monoamines, glutamate and GABA (Pitsillou et al., 2020). Although the pathogenesis of MDD is still poorly understood, it is well known that the interaction between genetic background and environment plays a critical role; in particular, stress is the major environmental risk factor for the development of MDD (Keers and Uher, 2011). Different studies on stress-based animal models and MDD patients have shown cellular and morphological alterations in different brain areas and have demonstrated that antidepressant treatments were able to revert these morphological defects (Duman, 2009; Licznerski and Duman, 2013; Rădulescu et al., 2021). However, traditional antidepressant drugs fail to induce a therapeutic effect in about 30% of patients with MDD, which are thus classified as having Treatment-Resistant Depression (TRD) (Al-Harbi, 2012).

Therefore, in this study, we differentiated induced pluripotent stem cells (iPSCs) derived from one healthy subjects (Bono et al., 2021) and two female TRD patients (Bono et al., 2020) into a mixed neuronal population, containing dopaminergic, serotonergic, GABAergic, glutamatergic and noradrenergic neurons, which was characterized both from a quantitative and morphological point of view. Then, iPSCs-derived neurons were treated with cortisol twice a day (9 a.m. and 5 p.m.) for three consecutive days, in order to mimic a stressful event (Mingardi et al., 2021), and stained for tyrosine hydroxylase (TH), glutamic acid decarboxylase 67 (GAD67) and vesicular glutamate transporter 1 (vGLUT1) through immunocytochemistry. Soma area was measured as a morphological parameter. Among our results, we showed that TRD patients presented an altered number of glutamatergic and GABAergic neurons and were also characterized by morphological defects in a patient-specific way. Moreover, an altered response to stress in TRD patients-derived dopaminergic neurons was observed. In conclusion, we observed that different TRD patients present specific morphological and quantitative alterations; thus, iPSCs represent a powerful tool for the study of patient-specific mechanisms underlying MDD and resistance to treatment.

References

- Al-Harbi KS. Treatment-resistant depression: therapeutic trends, challenges, and future directions. Patient Prefer Adherence. 2012;6:369-88. doi: 10.2147/PPA.S29716. Epub 2012 May 1. PMID: 22654508; PMCID: PMC3363299.
- Bono F, Mutti V, Piovani G, Minelli A, Mingardi J, Guglielmi A, Fiorentini C, Barbon A, Missale C, Gennarelli M. Generation of two human induced pluripotent stem cell lines, UNIBSi012-A and UNIBSi013-A, from two patients with treatment-resistant depression. Stem Cell Res. 2020 Dec;49:102104. doi: 10.1016/j.scr.2020.102104. Epub 2020 Dec 2. PMID: 33291010.
- Bono F, Mutti V, Piovani G, Minelli A, Mingardi J, Guglielmi A, Missale C, Gennarelli M, Fiorentini C, Barbon A. Establishment and characterization of induced pluripotent stem cell (iPSCs) line UNIBSi014-A from a healthy female donor. Stem Cell Res. 2021 Mar;51:102216. doi: 10.1016/j.scr.2021.102216. Epub 2021 Jan 29. PMID: 33548809.
- Duman RS. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. Dialogues Clin Neurosci. 2009;11(3):239-55. doi: 10.31887/DCNS.2009.11.3/rsduman. PMID: 19877493; PMCID: PMC3181922.
- Keers R, Uher R. Gene-environment interaction in major depression and antidepressant treatment response. Curr Psychiatry Rep. 2012 Apr;14(2):129-37. doi: 10.1007/s11920- 011-0251-x. PMID: 22198824.
- Licznerski P, Duman RS. Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression. Neuroscience. 2013 Oct 22;251:33-50. doi: 10.1016/j.neuroscience.2012.09.057. Epub 2012 Oct 2. PMID: 23036622; PMCID: PMC3566360.
- Mingardi J, La Via L, Tornese P, Carini G, Trontti K, Seguini M, Tardito D, Bono F, Fiorentini C, Elia L, Hovatta I, Popoli M, Musazzi L, Barbon A. miR-9-5p is involved in the rescue of stress-dependent dendritic shortening of hippocampal pyramidal neurons induced by acute

antidepressant treatment with ketamine. Neurobiol Stress. 2021 Aug 12;15:100381. doi: 10.1016/j.ynstr.2021.100381. PMID: 34458512; PMCID: PMC8379501.

- Pitsillou E, Bresnehan SM, Kagarakis EA, Wijoyo SJ, Liang J, Hung A, Karagiannis TC. The cellular and molecular basis of major depressive disorder: towards a unified model for understanding clinical depression. Mol Biol Rep. 2020 Jan;47(1):753-770. doi: 10.1007/s11033-019-05129-3. Epub 2019 Oct 14. PMID: 31612411.
- Rădulescu I, Drăgoi AM, Trifu SC, Cristea MB. Neuroplasticity and depression: Rewiring the brain's networks through pharmacological therapy (Review). Exp Ther Med. 2021 Oct;22(4):1131. doi: 10.3892/etm.2021.10565. Epub 2021 Aug 5. PMID: 34504581; PMCID: PMC8383338.

Primary prevention in pregnancy: a blending approach

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Preventive Medicine could be useful in Reproductive Health, offering sharing spaces about health education and doctor-patient communication with the aim of promoting protective behaviours.

During the visit for the DTaP vaccines administration or for flu vaccination the physician carries out a brief anamnesis about previous pregnancies, chronic pathologies, nutrition, drugs, smoking and alcohol intake in pregnancy. While the doctor gets the anamnesis, a nurse checks a series of clinical parameters and blood sugar level. At the time of administration of the vaccine a photo will be taken using the patient's smartphone and the pregnant woman will decide if post it on social-networks using some tags such as in the figure.

The post-vaccination observation time will be spent to offer some health messages to the patient. A scrollytelling of 10 minutes about pregnancy will be provided on a touchscreen tablet and a nurse will help the patient to memorize at least 5 key-messages about prevention and good practice for a successful pregnancy and baby health. The visit takes about 30 minutes and requires at least one physician and one nurse that must have adequate communication skills and be trained in vaccinology and reproductive health. The setting of the clinic must be clean, safe and comfortable and equipped with all the devices and m edications necessary to properly deal with emergency situations. The use of the patient’s smartphone and the publication of the photo by the patient herself relieve privacy issues.

The aim of this blending approach is to obtain several endpoints in a single step, such as information about food, drugs and supplements in pregnancy, possibility to have access to vaccines with transplacental transmission to the fetus, prevention of disease or disorder in pregnancy, advices in dangerous behaviors. So, each woman could have medical data of an unrecognized pregnancy hypertension or gestational diabetes.

Preventive Medicine needs a new scenario with structured interventions that not only involve the classical medical assistance but reach a large number of people through social media campaigns that can go viral and aims at the patient empowerment and raise o public awareness on reproductive health.

References

- COVID-19 vaccination hesitancy in Italian healthcare workers: a systematic review and meta-analysis. Bianchi FP, Stefanizzi P, Brescia N, Lattanzio S, Martinelli A, Tafuri S. Expert Rev Vaccines. 2022 Sep;21(9):1289-1300. doi: 10.1080/14760584.2022.2093723. Epub 2022 Jun 30. PMID: 35757890

Nanoparticle-mediated drug delivery of Helixcomplex snail mucus

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The Helixcomplex snail mucus is today used both for cosmetic and medical applications, and its efficacy in wound healing and mucoadhesivity was demonstrated in previous studies1,2,3. In nanoparticle (NP)-mediated drug delivery, liposomes are the most widely used drug carrier, and the only NP system currently approved by the FDA for clinical use, owing to their advantageous physicochemical properties and excellent biocompatibility4.

The aim of this work was to elucidate if was possible to use HC to carry out functionalized lipidic nanoparticles, such as Liposomes, and if their use could be useful to enhance its biological properties. The production of the lipidic nanoparticles was released using different concentration of HC; then biological compatibility of the so called “LipoHelix” was tested at different concentrations, from 10 to 50% to find the critical concentration within which the lipidic solution is uniform and stable. Their stability was monitored for the next 2 month. To evaluate the biological compatibility the lipidic nanoparticles were tested with an MTT assay.

LipoHelix was tested in vitro with a wound healing assay in comparison with the crude HelixComplex Snail Mucus and the ability to reconstitute the cellular monolayer after an artificial scratch evaluated after 24 hours. At the end of the experimentation the critical concentration observed was 50% of Helixcomplex snail mucus with an optimal effect of Lipohelix in would healing assay at 25%, with an effect double in comparison with HC.
In conclusion, these interesting results demonstrated how the use of nanoparticles can be the ideal application for HC in terms of biological compatibility, minor use of raw material and with the possibility to concentrate its actives obtaining a more focused action. Future works will regard the exploration of other biological properties and the processes at the base of the effects yet explored.

References

- Trapella C, Rizzo R, Gallo S, Alogna A, Bortolotti D, Casciano F, Zauli G, Secchiero P, Voltan R. HelixComplex snail mucus exhibits pro-survival, proliferative and pro-migration effects on mammalian fibroblasts. Sci Rep. 2018 Dec 5;8(1):17665. doi: 10.1038/s41598- 018-35816-3. Erratum in: Sci Rep. 2020 Jan 30;10(1):1834. PMID: 30518946; PMCID: PMC6281574.
- Gentili V, Bortolotti D, Benedusi M, Alogna A, Fantinati A, Guiotto A, Turrin G, Cervellati C, Trapella C, Rizzo R, Valacchi G. HelixComplex snail mucus as a potential technology against O3 induced skin damage. PLoS One. 2020 Feb 21;15(2):e0229613. doi: 10.1371/journal.pone.0229613. PMID: 32084249; PMCID: PMC7034816.
- Alogna A, Gentili V, Trapella C, Hallan SS, Sguizzato M, Strazzabosco G, Fernández M, Cortesi R, Rizzo R, Bortolotti D. Design of Liposomes Carrying HelixComplex Snail Mucus: Preliminary Studies. Molecules. 2021 Aug 4;26(16):4709. doi:
- 10.3390/molecules26164709. PMID: 34443296; PMCID: PMC8399803.
- Almeida B, Nag OK, Rogers KE, Delehanty JB. Recent Progress in Bioconjugation Strategies for Liposome-Mediated Drug Delivery. Molecules. 2020 Dec 1;25(23):5672. doi: 10.3390/molecules25235672. PMID: 33271886; PMCID: PMC7730700.

Antiviral effect of Quinoxaline Derivatives against Human Enteroviruses B and Bioinformatic RNA-Motif Identification as New Targets

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The Enterovirus genus includes many viruses that are pathogenic in humans, including Coxsackie viruses and rhinoviruses. Encephalitis, sepsis, poliomyelitis, acute heart failure, and myocarditis are typical manifestations of enterovirus infections in humans. Currently, effective antiviral agents are not available for the treatment or prevention of enterovirus infections, which remain an important threat to public health.

We investigated the susceptibility of a panel of representatives Enterovirus (enterovirus A71, belonging to A species; coxsackieviruses B4 and B3; echovirus 9, belonging to B species; and enterovirus D68, belonging to D species) to quinoxaline derivatives at non-toxic concentrations. Preliminary assays were performed to assess the antiviral power of the compounds such as the plaque reduction assay and MTT method to evaluate the inhibition of virus-induced cytopathogenicity, and the relative yield reduction. Then we investigated ability of compounds to directly disrupt viral particles and step of virus replication hijacked by a time-of-drug addiction assay at defined time point. In addition, a bioinformatic analysis was carried out to discover potential new conserved motifs in CVB3 and CVB4 compared to the other enterovirus species that can be used as new targets.

We identified interesting anti-enterovirus B agents with significant antiviral activities. Derivatives 6, 7, and 8 exhibited the highest inhibitory activity by interfering with virions entry into the host cell mediated by the VP1 capsid protein. Parallelly, we ran a bioinformatics analysis to discover potential new motifs in all the enterovirus species. The analysis revealed potential antiviral target on mRNA shared by only CVB3 and CVB4. In silico prediction of RNA–protein interactions prompted us to hypothesize that changes in this target may have an additive effect at the level of mRNA splicing, thus causing a reduction in virus replication.

Among enteroviruses, coxsackievirus B results in medically important pathogens related to spastic paralysis. Although selected quinoxaline derivatives inhibited CVB3, CVB4, and E9 infections, with EC50 values in the low micromolar range, our findings on the potential mechanism of action need further investigation to develop more interesting derivatives or combination treatments.

References

- Cherry J., Demmler-Harrison G., Kaplan S., Steinbach W., Hotez P. Feigin and Cherry's Textbook of Pediatric Infectious Disease. 8th ed. Elsevier; Amsterdam, The Netherlands: 2017. pp. 1449–1542.

The pharmacological blockade of mGluR5 with CTEP improves disease course in SOD1G93A ALS animal model

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, with no effective cure, characterized by death of upper and lower motor neurons (MNs). Although the aetiology is still unclear, glutamate (Glu)-mediated excitotoxicity plays a pivotal role. In this scenario glutamatergic receptors, in particular Group I metabotropic glutamate receptors (mGluRs) represent a druggable target. Consistently, we recently demonstrated that SOD1G93A mice, a widely used animal model of ALS, carrying a partial or total genetic deletion of mGlu5 receptor showed a delayed disease onset, prolonged survival probability, accompanied by a significant MNs preservation and reduction of glial activation.

We investigated the effect of the selective mGluR5 pharmacological blockade with the orally available negative allosteric modulator CTEP, in ALS mice. Male and female animals were chronically administered with CTEP (2 mg/kg every 48 hours or 4 mg/kg every 24 hours) by gavage, starting at 90 days of life (as early symptomatic stage) until the human endpoint. Disease progression was monitored by in-vivo functional studies and ex-vivo immunohistochemical (IHC) analyses.

CTEP-treatment demonstrated both in-vivo and ex-vivo beneficial effects. Concerning the in-vivo studies, CTEP treatment dose dependently slowdown the progression of the pathology, ameliorated functional outcomes and increased the survival probability, in SOD1G93A mice. The lower dosage showed significant results in female mice only, while the higher dose of CTEP demonstrated significant therapeutic outcomes in both male and female SOD1G93A mice compared to controls. Ex-vivo analyses showed decreased astrocyte and microglia activation, preserved MN death, and a reduction in the excessive glutamatergic neurotransmission, in the spinal cord of both male and female CTEP(4 mg/kg)-treated mice. Pharmacokinetics did not detect sex-significant differences in CTEP concentration measured in blood, liver, and CNS tissues (1).

Our results demonstrate that the pharmacological blockade of mGlu5 receptors, produces beneficial effects in the SOD1G93A mouse model of ALS. The in-vivo effects can be ascribed to a reduced reactive gliosis that contributes to preserve MN. Our pre-clinical evidence suggests mGluR5 as a promising target highlighting the application of mGluR5 modulators as favorable pharmacological tools that can be tested in clinical trials for ALS treatment.

References

- Milanese M, Bonifacino T, Torazza C, Provenzano F, Kumar M, Ravera S, Zerbo AR, Frumento G, Balbi M, Nguyen TPN, Bertola N, Ferrando S, Viale M, Profumo A, Bonanno G. Blocking glutamate mGlu5 receptors with the negative allosteric modulator CTEP improves disease course in SOD1G93A mouse model of amyotrophic lateral sclerosis. Br J Pharmacol. 2021 Sep;178(18):3747-3764. doi: 10.1111/bph.15515. Epub 2021 Jun 29. PMID: 33931856; PMCID: PMC8457068.

Hepatitis B Virus prevalence and serological profiles in a hospital in Southern Italy

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Viral hepatitis still represents a significant worldwide public health issue, being an important cause of morbidity and mortality. Hepatitis B virus (HBV) and Hepatitis C virus are among the leading causes of chronic liver diseases such as cirrhosis and hepatocellular carcinoma [1] [WHO, 2021]. Hepatitis B can be diagnosed by serological dosage of specific viral markers like proteins produced by the virus or antibodies produced by the host [2]. Since 1991, HBV vaccination became mandatory [3]. The aim of our study is to evaluate the HBV markers prevalence from serologic analysis of hospitalized patients at University Hospital of Campania "Luigi Vanvitelli" between January 2020 to December 2020.

We screened serum hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), antibody to hepatitis B core antigen (anti-HBc) (Ortho Clinical Diagnostics VITROS® 3600 Immunodiagnostic System, Rochester, NY, USA). For the purposes of this analysis, positive results for anti-HBc indicate past or ongoing HBV infection, while positive results for anti-HBs only indicated immunity vaccination or past infection. Negative patients for analyzed HBV serological markers were considered to be non-immune or non-infected.

Of the total of 1816 patients serological data showed 1.5% HBsAg positive subjects, 27% anti-HBc and 39.4% anti-HBs positive subjects. According to the serological profile, subjects were divided into subgroups to evaluate infection stages, vaccinated subjects and NO markers population. 394 patients (21.7%) were anti-HBs positive, while 51.3% of the study population showed NO markers and so were susceptible to infection [Fig. 1].

The current HBV seroprevalence situation demonstrates the need for continuously improved vaccination because of lower immunogenicity in adolescence or adulthood. Immunological memory persists for years after HBV vaccination but there remains the possibility that individuals vaccinated in childhood presenting a low anti-HBs titre and could be infected. Estimation of antibody titre 5 and 10 years post-vaccination determines the need for a booster dose in subjects over 20. Our findings reaffirm the importance of health surveillance in hospitalized subjects and the need to improve vaccination rates to limit the number of non-immunized subjects in order to increase the general population's health.

References

- Lavanchy D. (2005). Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology, 34 Suppl 1, S1–S3.
- Dienstag JL. (2008). Hepatitis B virus infection. The New England journal of medicine, 359(14), 1486–1500.
- Mele A, Tosti ME, Mariano A, Pizzuti R, Ferro A, Borrini B, Zotti C, Lopalco P, Curtale F, Balocchi E, Spada E. (2008). Acute hepatitis B 14 years after the implementation of universal vaccination in Italy: areas of improvement and emerging challenges. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 46(6), 868–875

Generation of a new AIF peptide targeting Human Cyclophilin A to inhibit AIF-mediated cell death

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Apoptosis inducing factor (AIF) and cyclophilin A (CypA) complex formation play a crucial role in neuronal cell loss following several apoptotic stimuli.[1] Blocking this interaction through an AIF-based synthetic fragment, covering residues 370-394 (AIF(370- 394)), induces neuroprotection in neuronal cell models of glutamate-induced neurotoxicity and prevents brain injury in neonatal mice after hypoxia-ischemia, showing that AIF/CypA complex is a significant candidate for generating pharmacological inhibitors to block the cell death process.[1,2] However, to date pharmaceutical compounds targeting this complex are missing.

Peptide was prepared by Fmoc solid-phase peptide synthesis (SPPS). Peptide structures in solution and in complex with CypA were studied by Nuclear Magnetic Resonance (NMR) and molecular modeling. The ability of peptide to interact with CypA was evaluated by using Epic Corning label free technique. Neuroprotective effects of peptide against Glutamate-Induced HT-22 Hippocampal Cell Damage were evaluated by MTT assays.

New AIF-based bioactive peptide was identified. It spans residues 381-389 of AIF (AIF(381-389)) and is cyclized through a disulfide bond connecting two cysteines added at the C- and N-terminal ends of the chain, in order to stabilize the native-like beta-hairpin structure.[3] Notably, despite small size of the new peptide, less than half of the precursor, it shows a similar in vitro affinity for CypA, an enhanced proteolytic stability and an improved antiapoptotic activity in neuronal cells compared to the parent peptide, without apparent cytotoxic effects.[3] Furthermore, NMR-based 3D model of the AIF(381-389)/CypA complex provided a better understanding of the binding hot spots on both the peptide and the protein and provided evidences on the significant role of the backbone conformation on the biological activity of the peptide.

The obtained structural data are very useful for drug development programs based on structure-activity-relationship studies and on computational approaches to improve peptide activity and to predict new highly effective, selective, stable inhibitors.

References

- Doti N, Reuther C, Scognamiglio PL, et al. (2014) Cell Death Dis, 5,e993.
- Zhu C, Wang X, Deinum J et al. (2007) Exp Med, 204(8):1741-1748.
- Russo L, Mascanzoni F, Farina B, et al. (2021) J Med Chem, 64(15):11445-11459.

Cupferron as antimicrobial compound against Hospital-Acquired Escherichia coli infections

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Escherichia coli (E. coli) infections are a clinical problem in both community and hospital settings. Evaluation of the disease burden due to antimicrobial resistant Escherichia coli infections highlights the urgent need for new therapeutic strategies to mitigate the spread of antibiotic resistance. In this context, the aim of study was to evaluate the antibacterial activity of N-nitroso-N-phenylhydroxylamine ammonium salt (Cupferron) against multisensitive standard and clinically isolated strains E. coli producing extended-spectrum beta-lactamases and resistant to carbapenems.

Cytotoxicity was evaluated by 3- [4,5-dimethylthiazol-2-yl] -2,5 diphenyl tetrazolium bromide (MTT) on VERO-CCL81 cell line and hemolysis assays in a concentration range of 400 – 1.56 µg/mL. The antibacterial activity was assessed by the disk diffusion, plate microdilution and time killing tests. Moreover, its impact against biofilm and catalase virulence was estimated through biofilm attachment/inhibition/degradation and catalase tests, respectively.

Cupferron represents a high-performance antibacterial compound. The inhibitory area on Mueller Hinton agar plates was in the range of 16-18 mm for E. coli ATCC 11229 and clinical isolates. The minimum inhibitory concentration (MIC) of Cupferron, exerting bacteriostatic action on 90% of planktonic E. coli (MIC90), was 200 and 100 µg/mL for multidrug-resistant and sensitive strains, respectively. The compound impacted the different stages of biofilm formation, exhibiting 33 and 42% of inhibition after 2 and 24 hours, and 45% of mature biofilm eradication, against E. coli ATCC 25922. Moreover, Cupferron-treated bacteria showed increased susceptibility to oxidative stress, detecting a fold-increase of 1.27 and 1.20 at ½ and ¼ MIC values. The active concentrations of Cupferron did not affect VERO-CCL81 and erythrocytic cell viability.

Together, these findings, suggested the Cupferron could represent a promising candidate for tackling E. coli infections.

References

- Bai, Y.; Wang, W.; Shi, M.; Wei, X.; Zhou, X.; Li, B.; Zhang, J. Novel Antibiofilm Inhibitor Ginkgetin as an Antibacterial Synergist against Escherichia coli. Int. J. Mol. Sci. 2022, 23, 8809.
- Ruan, X., Deng, X., Tan, M. et al. In vitro antibiofilm activity of resveratrol against avian pathogenic Escherichia coli. BMC Vet Res 17, 249 (2021).
- European Antimicrobial Resistance Surveillance Network (EARS-Net) reporting protocol for 2021 (https://www.ecdc.europa.eu/en/publications-data/ears-net-reporting-protocol-2022)