In this context, we have identified a new promising candidate: Topoisomerase II alpha, a protein responsible for resolving of the DNA catenation both in the DNA replication and mitosis. Defective localisation of Topoisomerase II alpha may contribute to the observed mitotic defects in these cells. We are currently exploring the impact of these defects on brain development using reprogramming techniques to assess proper neuronal differentiation.

P5 - TRANSLATIONAL CONTROL OF Δ160P53 KEEPS THE DARK SIDE OF TP53 IN CHECK

Ana Catarina Ramalho[1,2], Rafaela Lacerda[1], Luísa Romão[1], Marco M. Candeias[1,2]

[1]Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal. [2] MaRCU – Molecular and RNA Cancer Unit, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

The TP53 tumour suppressor gene was discovered over 40 years ago, but to this day some aspects of its regulation and function remain a mystery. It encodes the full-length p53 protein (FLp53), a transcription factor with a key role in stress response in multicellular organisms, that can either direct cells towards apoptosis or recovery of homeostasis. With such a decisive role, its expression and activity are tightly regulated. A vast set of RNA-binding proteins (RBPs) have been described to affect the translation of FLp53 or the stability of p53 mRNA in response to different perturbations. But in addition to FLp53, there is a group of shorter protein isoforms lacking the N-terminal region, which have well-described functions, and are translated from the same mRNA. The shorter and less studied isoform is Δ160p53, which promotes cell survival, proliferation, and invasion. Despite its usual low levels, it is commonly overexpressed in tumours. However, the detailed mechanisms and factors involved in the regulation of Δ160p53 are still unknown.

In this work, a mass spectrometry was performed to identify the proteins in an RNA-protein co-immunoprecipitation of the p53 mRNA using the MS2 system in the p53-null cell line H1299. The validation of the hits was undertaken by western blot with specific antibodies after immunoprecipitation. The effect of the binding proteins on the translation of Δ160p53 was assessed by overexpression or knockdown, and the expression levels were verified by western blot or luminescence assays.

The mass spectrometry allowed the identification of potential new binding partners of the p53 mRNA. Resorting to the literature and to computational tools available online to predict protein-RNA interactions, a few hits were selected for follow-up and their interactions confirmed. Simultaneously, the modulation of Δ160p53 expression by some of these proteins was verified.

Considering the importance of TP53 in deciding the fate of the cell, the observation of abnormal levels of the oncoprotein Δ160p53 in cancer is intriguing. Understanding the control of its translation could uncover strategies to block it and pave way for new cancer therapies.

P6 - THE Y-CHROMOSOMAL HAPLOTYPE DIVERSITY OF R1B-M269 SUBHAPLOGROUPS IN INDIVIDUALS FROM THE CENTRAL REGION OF PORTUGAL

Fábio Nunes[1], Licínio Manco[1]

[1]Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Introduction: The most frequent Y-chromosomal haplogroup in the Iberian Peninsula is R1b-M269, representing frequencies of about 60% in Portugal. The R1b-M269 splits into geographically localized subhaplogroups, showing the S116-DF27 branch as the most common in the Iberian Peninsula (40-48%) [1]. We have previously shown that the subhaplogroup DF27 was the most common (70%) among R1b-M269 individuals from Central Portugal [2]. This study aimed to analyze the distribution of the Y-chromosomal haplotypes within the main branches of the R1b-M269 haplogroup in individuals from the central region of Portugal.

Methodology: The study sample comprised 54 individuals carrying the derived allele at the M269 SNP from districts of Aveiro, Coimbra, Guarda and Viseu. The internal variation of R1b-M269 samples was assessed by analyzing seven Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393). Multiplex PCR reactions were performed using the QIAGEN Multiplex PCR kit. The forward primers were labeled with Cy5 on the 5' end. The PCR fragments were analysed on an automate ALFexpressTM II sequencer (Amersham Biosciences). The frequencies of the Y-STR haplotypes were determined by direct counting. Population pairwise genetic distances (RSTs) and p-values were calculated using the software Arlequin v3.5.

Results and Discussion: The gene diversity of the seven markers ranged from 0.143 (DYS393) to 0.558 (DYS389II) for the sample set. The overall haplotype diversity was 0.343, similar to other Iberian populations (mean 0.368 for five Iberian populations [3]). The total number of observed haplotypes was 31, the most frequent (0.148) was compatible with the "Atlantic Modal Haplotype" (AMH) (DYS19-14/ DYS390-24/ DYS391-11/ DYS392-13/ DYS393-13). The network constructed with the 31 different haplotypes produced a star-like structure with a center occupied by the most common AMH-compatible haplotype (14-13-29-24-11-13-13), shared by eight samples and four different R1b-M269 suclades. The network showed only one missing link, which could indicate evolutionary histories of the R1b-M269 paternal lineages inside the region. The genetic distances between six Iberian populations showed no significant differences, except between Portugal and the Native Basques (RST=0.007; P=0.054).

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P7 - A 3D CELL CULTURE MODEL OF THE TUBERCULOSIS GRANULOMA THAT CAN BE APPLIED FOR HOST GENETIC STUDIES IN THE CONTEXT OF A MULTICELLULAR IMMUNOLOGIC RESPONSE TO INFECTION

Susana David[1,2], Manoj Mandal[2], Elsa Anes[2], David Pires[2,3]

[1] Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisbon, Portugal. [2] Host-Pathogen Interactions Unit, Research Institute for Medicines, iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. [3] Universidade Católica Portuguesa, Católica Medical School, Center for Interdisciplinary Research in Health, Rio de Mouro, Portugal.

Introduction: The granuloma is an inflammatory infiltrate of mononuclear cells. Some bacterial infections are characterized by the formation of granulomas as part of the immune response to contain the infection. Granuloma models have contributed valuable insights into the genetic basis of granuloma formation during infection. For example, IFNGR1 and IFNGR2 variants have been found to disrupt the immune response, resulting in impaired granuloma formation and increased susceptibility to diseases by Mycobacterium sp. More easily implemented comprehensive models would facilitate the study of the different immune mechanisms and help identify new disease-associated genes. Our objective is to generate an in vitro 3D cell culture model using human primary cells and microspheres to generate a stratified granuloma model for future use in genetic, immunological and drug discovery studies.

Methods: A commercial system was used to encapsulate human peripheral blood mononuclear cells (PBMC) infected with GFP-expressing M. tuberculosis and maintained in culture for several weeks. The cellular constituents of these granulomas and their organization were characterized by fluorescence microscopy and flow cytometry as well as the viability of the cells and the extent of bacterial replication in factor of time.

Results: The results demonstrate a ready recruitment of cells towards infected macrophages, leading to the formation of densely populated aggregates. These aggregates maintained cell viability for several weeks and displayed an enhanced control of bacterial replication compared to the more common monolayer infection models. Moreover, the capsules can be easily disrupted when required to isolate genetic material for further analysis.

Conclusion: The proposed 3D model resembles some structural and cellular characteristics of the tuberculosis granuloma and maintains its stability beyond more common 2D models of infection. These preliminary results demonstrate that this model can be used to further explore the determinants of granuloma formation and host response to infection.

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