PS185

Lung branching morphogenesis, in the chicken model, is accompanied by temporal metabolic changes

H. Fernandes-Silva 1,2,∗, M.G. Alves 3,4, J. Correia-Pinto 5,6, P.F. Oliveira 5,6,7, R.S. Moura 1,2
1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, 4710-057 Braga, Portugal
2 ICVS/3B’s – PT Government Associate Laboratory, 4710-057 Braga/Guimarães, Portugal
3 Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal
4 Department of Microscopy, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, 4050-313 Porto, Portugal
5 Department of Pediatric Surgery, Hospital de Braga, 4710-243 Braga, Portugal
6 13S – Instituto de Inovação e Investigação em Saúde, University of Porto, 4050-313 Porto, Portugal
7 Department of Genetics, Faculty of Medicine (FMUP), University of Porto
E-mail address: hugomiguelfsilva@gmail.com (H. Fernandes-Silva).

Aim: In this work, we characterized, for the first time, the metabolic profile of chick lung branching in early stages of development: b1, b2 and b3 (1, 2 or 3 secondary bronchi, respectively).

Introduction: Pulmonary development is a complex process that depends on the activation of conserved signaling pathways that regulate cellular processes such as proliferation, differentiation and migration.1−3 These cellular processes require high amounts of energy and nutrients to form new biomass.4,5 However, the metabolic changes that occur during lung branching morphogenesis have not been described so far.

Methods: Ex vivo lung explant culture was performed and the medium collected to analyze the production/consumption of metabolic intermediates associated with glucose catabolism (lactate, acetate, alanine), by 1H-NMR. qPCR was performed to assess the expression levels of key enzymes and transporters from the correspondent metabolic pathways.

Results: The results showed that the major variations occur from stage b1 to stage b3. In b3 there is an increase in lactate and acetate production. Still, glucose consumption is maintained from b1 to b3 stage, with a concurrent decrease of glucose transporter 3 (glut3) transcript levels. Hexokinase 1 (hk1) levels also decrease in b3 stage (as compared to b2). This phenomenon suggests an increase in the glycolytic efficiency and a shift to lactic acid production (in detriment of mitochondrial respiration). In fact, we observed a decrease on pyruvate dehydrogenase B (pdhB) and an increase in lactate dehydrogenase A (ldhA) expression levels in b3 stage (as compared to b2), while lactate dehydrogenase B (ldhB) levels decrease.

Conclusion: This study describes, for the first time, the temporal metabolic changes associated with chick pulmonary branching. It seems that glycolytic efficiency is increased and Krebs cycle metabolism shifts to lactate production along development. Furthermore, acetate and lactate are potentially seen as metabolic biomarkers of lung development.
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References

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PS186

Epigenetic modifications as targets to new therapies for Chronic Lymphocytic leukaemia – A preliminary study

B. Ribau 1,2,∗, J. Jorge 2,4, R. Alves 2,3, P.I. Ribeiro 2,4, A.C. Gonçalves 2,3,4, I.M. Carreira 4,5, A.B. Sarmento-Ribeiro 2,3,4,6
1 Department of Chemistry, University of Aveiro, Portugal
2 Laboratory of Oncobiology and Hematology (LOH), University Clinic of Hematology and Applied Molecular Biology, FMUC, Portugal
3 Center for Neuroscience and Cell Biology, IBILI (CNC.IBILI), University of Coimbra, Portugal
4 CIMAGO - Center of Investigation on Environment Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Portugal
5 Laboratory of Cytogenetics and Genomics (LCG), Faculty of Medicine, University of Coimbra, Portugal
6 Clinical Hematology Service, University Hospital of Coimbra, Portugal
E-mail address: beatriz.ribau@ua.pt (B. Ribau).

Aim: This study aimed to clarify the involvement of epigenetic modifications in chronic lymphocytic leukaemia development and analyse the therapeutic potential of epigenetic modulators.

Introduction: CLL is the most common type of leukaemia found in adults and is an extremely variable and heterogeneous disease. The CLL aetiology is unknown and it natural history is heterogeneous. However, epigenetic modifications may play an important role in CLL.

Methods: This study enrolled 18 CLL and 7 controls. To perform primary CLL cultures, peripheral blood mononuclear cells from CLL patients were isolated using ficoll gradient and incubated with the hypometilants, Azacytidine and Decitabine, and deacetylase inhibitors, Panobinostat and Vorinostat, in monotherapy (single dose and daily administration) and in combination for 24h/48h. The cytotoxic/cytostatic effect of drugs was evaluated by fluorometric microculture cytotoxicity assay (FMCA). Cell death and cell cycle were determined by flow cytometry using Annexin V and PI/RNAsé, respectively. CDS and CD19 antibodies were used to identify normal (CD5−/CD19+) and neoplastic cells (CD5+/CD19+). Methylation pattern was determined by MS-MLPA. Data were analysed using univariate approaches.

Results: Preliminary results show that patients appear to be more sensitive to Azacytidine and Vorinostat than Decitabine and Panobinostat, on single dose administration. Combination of Panobinostat with Azacytidine and Decitabine induced higher cytotoxicity than single dose. For all drugs, daily administration schedule reduced more effectively cell viability/proliferation than the same doses in single administration. These drugs induced cell death mainly by apoptosis with specificity to neoplastic cells. Moreover, CLL patients had a significant higher methylation frequency of PAX5 (70%), KLLN (80%), WT1 (100%), THBS1 (90%) and GATA5 (90%) gene promoters when compared with controls (all genes demethylated, except MSH6). Furthermore, all CLL patients had at least one methylated gene.

Conclusion: The preliminary results suggest that methylation of tumour suppressor genes is a common event in CLL patients and that epigenetic modulators induce a cytotoxic effect, reducing cell viability/proliferation, in a time- and dose-dependent manner. Therefore, these results are promising and encourage further studies in CLL.

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PS191

Imaging features of brain metastases from testicular cancer

Ana Filipa Pinto 1,∗, Susana Maria Silva 2,3, Eduarda Carneiro 4, Diana Ferreira 4, Joaquina Maurício 5, Mavilde Arantes 2,3,4
1 Faculty of Medicine of the University of Porto, 4200-319 Porto, Portugal
2 Unit of Anatomy, Department of Biomedicine, Faculty of Medicine of the University of Porto, 4200-319 Porto, Portugal
3 Center for Health Technology and Services Research (CINTESIS), 4200–450 Porto, Portugal
4 Division of Neuroradiology, Radiology Service, Portuguese Institute of Oncology, Porto, Portugal
5 Medical Oncology Service, Portuguese Institute of Oncology, Porto, Portugal
E-mail address: anafilipapinto95@gmail.com (A.F. Pinto).

Aim: Our study evaluated the incidence, imaging characteristics, and prognosis of brain metastases originating from primary testicular tumors.

Introduction: Approximately 95% of testicular tumors are testicular germ cell tumors (TGCT).1 Sertoli cell tumors are rare non-germ cell origin tumors and account for less than 1% of testicular cancer.2 Brain metastases from germ cell tumors are very uncommon, occurring in less than 2–3% of patients.3 In testicular cell cancer, it is estimated that the incidence of brain metastases is 1–2% in all TGCT, whereas in advanced stages of TGCT the incidence rises to about 10–15% 4–9

Methods: Case records of testicular tumors patients within the IPO Porto data base from 2006 to 2015 were reviewed to identify patients with testicular tumors and evidence of brain metastases.

Results: 368 patients with testicular tumors were identified, with only four having evidence of brain metastases.