GE11 positive exosomes as a potential RNAi delivery system in clear cell Renal Cell Carcinoma

B. Adem 1,*, A.L. Teixeira 2,4, F. Dias 2,3, C. Ruivo 1, R. Medeiros 2,3,4,5,*, S.A. Melo 1,5,*  

1 Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (ISU), 4200 Porto, Portugal. Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), 4200 Porto, Portugal.  
2 Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), 4200-072 Porto, Portugal.  
3 LPCC, Research Department Portuguese League Against Cancer (Liga Portuguesa Contra o Cancro-Núcleo Regional do Norte), 4200-177 Porto, Portugal.  
4 Health Sciences Faculty, Fernando Pessoa University, 4249-004, Porto, Portugal.  
5 FMUP, Medical Faculty of the University of Porto, Portugal.  

E-mail address: badem@ipatimup.pt (B. Adem).

Aim: Use GE11 positive (GE11+) exosomes as a targeted delivery system to EGFR overexpressing cells for the treatment of clear cell Renal Cell Carcinoma (ccRCC).

Introduction: ccRCC is the most prevalent subtype of renal cancer and the most lethal urologic tumor. Generally, it is radiochemotherapy resistant, and frequently associated with relapse after 5–11 months upon targeted therapy treatment, which highlight the need to develop new therapeutic strategies. Exosomes, extracellular vesicles of 40–150 nm that mediate intercellular communication, have emerged as promising therapeutic tools due to their engineering potential and ability to evade the immune system.

Methods: EGFR is known to be overexpressed in ccRCC thus, the expression of GE11, a peptide that binds to EGFR, in exosomes membrane enable a targeted delivery of therapeutic molecules to EGFR overexpressing cells. Exosomes derived from HEK293T were engineered to express the GE11 peptide on their surface and incubated with normal or tumor renal cell lines.

Results: Our results revealed EGFR overexpression at the mRNA and protein levels in a ccRCC cell line, compared to a normal renal cell line. Furthermore, tumor cells presented increased protein levels of phosphorylated EGFR when compared to normal cells. These results support the hypothesis of using an EGFR-based exosomes delivery model, the GE11+ exosomes. A higher percentage of tumor cells internalized GE11+ exosomes compared to exosomes derived from HEK293T cells transfected with control condition. Additionally, tumor cells exhibited an increased mean of fluorescence intensity compared to the control, suggesting that each cell uptakes more GE11+ exosomes in an EGFR-dependent manner. Importantly, GE11+ exosomes were internalized in a greater proportion by tumor cells rather than normal renal cell lines.

Conclusion: Overall, the use of GE11+ exosomes as a new delivery system is promising therapeutic strategy for ccRCC treatment. Ultimately, these exosomes can be loaded with RNAi-based drugs to target deregulated genes in ccRCC.

Evaluation of combined cytoplasmic AR in tumour cells expression and tumour CD3 T-cells infiltrate as a prognostic score for patients with prostate cancer

V. Constâncio 1,2,*, M. McAllister 2, S. Patek 2, M. Underwood 3, H. Leung 4, J. Edwards 2  

1 Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, United Kingdom  
2 Department of Urology, Queen Elizabeth University Hospital, Glasgow, United Kingdom  
3 Beatson Institute of Cancer Research, United Kingdom  
4 E-mail address: veraconstancio@ua.pt (V. Constâncio).

Aim: We aimed to assess the prognostic value of using a cumulative score evaluating the expression of Androgen Receptor (AR) and the presence tumour inflammatory infiltrate as a prognostic marker for prostate cancer (PCa).

Introduction: PCa is the most common male cancer, in Europe. Currently, at diagnosis, only tumour-based factors, including clinical stage, tumour grade and circulating concentrations of Prostate-Specific Antigen (PSA) are used to predict PCa outcome. However, this can vary within patients sharing the same clinical conditions, leading to patient’s over/under treatment. It is now recognized that cancer progression is also dependent on tumour’s interaction with its microenvironment, specifically with immune cells. Therefore, the development of predictive biomarkers, capable of combining these two factors should be considered.

Methods: Immunohistochemistry for AR expression and CD3 T-cells was performed on biopsies from a cohort of 361 patients diagnosed with PCa. Semi-quantitative weighted histoscore and quantitative assessments were used.

Results: High cytoplasmic AR expression in tumour cells and high CD3 T-cells presence were associated with reduced overall survival (p=0.000055, and p=0.004, respectively), with strong association (p=0.001) on X2 analysis. When patients were grouped as having both markers low or one low and low/moderate and one high, and both high, this cumulative prognostic score was strongly associated with overall survival (p=0.000001), being the mean overall survivals: 7.1 years (95% CI 6.5–7.6), 6.0 years (95% CI 5.4–6.6) and 3.8 years (95% CI 2.4–5.0), respectively. Moreover, on multivariate analysis, it was considered a significant independent predictor of overall survival (HR 1.982, 95% CI 1.018–3.859, p = 0.044).

Conclusion: These results confirm the clinical utility of assessing both tumour and microenvironment characteristics when predicting patients’ outcome, and suggest that the presence...
of high cytoplasmic AR expression in tumour cells and CD3 T-cells predicts poor outcome for patients diagnosed with PCa.

http://dx.doi.org/10.1016/j.pbj.2017.07.017

PS165

ALDHs as potential biomarkers in myeloid neoplasms – Preliminary study

B. Macedo 1, 2, J. Jorge 2, 3, R. Alves 2, 3, A.C. Gonçalves 2, 3, 4, A.B. Sarmento-Ribeiro 2, 3, 4, 5

1 Department of Chemistry, University of Aveiro, Portugal
2 Laboratory of Oncobiology and Hematology, University Clinic of Hematology and Applied Molecular Biology, Faculty of Medicine, University of Coimbra, Portugal
3 Center for Neuroscience and Cell Biology, IBILI, University of Coimbra, Portugal
4 CIMAGO – Center of Investigation on Environment Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Portugal
5 Clinical Hematology Service, University Hospital of Coimbra, Portugal

E-mail address: barbaramacedo@ua.pt

(B. Macedo).

Aim: The aim of the study is to evaluate the expression of aldehyde dehydrogenase (ALDH) in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) to verify their potential as a marker for the diagnosis and/or prognosis of these diseases.

Introduction: ALDH superfamily is a group of 19 enzymes critical to the protection against toxic aldehydes, and have been associated with multiple diseases, namely in cancer. MDS are characterized by ineffective hematopoiesis associated with progressive peripheral blood cytopenias, and a predisposition toward leukemic transformation. MDS pathophysiology is a complex multistep process that involves genetic and epigenetic abnormalities in genes associated with differentiation, cellular proliferation, and apoptosis. Since ALDHs are involved in some of these biological processes, the deregulation of these enzymes may influence MDS and AML development.

Methods: To this end, we analyzed the expression levels of 8 ALDH isoforms, ALDH1A1, ALDH1A2 ALDH1B1, ALDH1L1, ALDH1L2, ALDH3A2, ALDH4A1, and ALDH16A1, in 31 patients (16 MDS and 15 LMA) and 19 healthy controls. ALDH expression levels were analyzed using RT-PCR and differentially expressed genes were quantified by qPCR. The statistical analysis was carried out by variance analysis and χ2 test. Survival were analyzed by Kaplan Meier curves (p < 0.05).

Results: Preliminary results indicate that all MDS patients express ALDH16A1 isoform whereas only 67% of controls (p < 0.05) show expression of this isoform. Moreover, AML patients have lower ALDH1A2 expression levels than MDS and controls and only 20% of AML patients express this isoform (MDS = 54% and controls = 55%). The ALDH1L2 is only expressed in chronic myelomonocytic leukemia subtype of MDS. Furthermore, the expression of ALDH isoforms does not appear to influence patient overall survival.

Conclusion: According to these results, ALDH isoforms have differential expression patterns in MDS and AML patients when compared with controls and each other. Further studies are needed to prove their potential as a diagnostic/prognostic biomarkers.

http://dx.doi.org/10.1016/j.pbj.2017.07.018

PS155

Discovery of novel mechanisms of centrosome amplification and their therapeutic value in cancer

B.P. de Almeida 1, 2, *, G. Marteil 3, M. Bettencourt-Dias 5, N.L. Barbosa-Morais 2

1 Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Faro, Portugal
2 Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
3 Instituto Gulbenkian de Ciência, Oeiras, Portugal

E-mail address: bernardo.almeida@medicina.ulisboa.pt

(A. de Almeida).

Aim: To understand the mechanisms of centrosome amplification and their therapeutic value in cancer.

Introduction: Centrosomes are the major microtubule-organising centres of animal cells. Centrosome amplification (CA) – the presence of more than two centrosomes in a cell – is a common feature in cancer and was recently shown to be sufficient to drive tumorigenesis. Recent work from the Bettencourt-Dias Lab has identified a new recurrent feature of cancer cells: centriole over-elongation, which also promotes CA. However, origins of those abnormalities and their therapeutic value remain poorly understood.

Methods: We have screened the NCI-60 panel of human cancer cell lines for centriole number and individual length to test their frequency and interdependence. We have thereby also generated a metric capturing each abnormality level per cell line that we then correlated with the publicly available molecular (e.g. genomic, transcriptomic and proteomic) and drug-sensitivity quantitative profiles for that panel.

Results: Our single-centriole analyses showed that longer centrioles are more common in cells with CA and that cells do not control their overall centriolar mass when the centriole number increases. Moreover, cancer cell lines with longer centrioles proliferate slower due to an accumulation of cells in G1 phase, suggesting that centriole length defects could lead to a cell cycle delay in G1. In addition, our original genome-wide approach highlighted putative mechanisms associated with susceptibility to both abnormalities, such as the proteasome protecting cells from CA. Correlation with drug activity identified some compounds as potential therapeutic options to selectively target cells with higher incidence of centriole abnormalities.

Conclusion: This work provides the first single-centriole-level portrait of centriole abnormalities in cancer and contributes to the understanding of their molecular origins, namely by revealing novel molecular mechanisms in cell cycle biology. Given the cancer-specificity of these abnormalities, the identified compounds will inspire the development of drugs to selectively target cancer cells.

Acknowledgements: This work is supported by an EMBO Installation Grant to NLBM.

References


http://dx.doi.org/10.1016/j.pbj.2017.07.019