Eur Respir Rev 2008; 17: 107, 49–50
DOI: 10.1183/09059180.00010707

VIRAL INFECTION DRIVES TISSUE FIBROSIS *IN VITRO*

Andrea P. Malizia*,¶, Dermot Walls#, Jim J. Egan¶ and Peter P. Doran*

Cell and Molecular Biology Young Scientist Travel Award, sponsored by AstraZeneca

*School of Medicine and Medical Science, University College Dublin, Dublin, Ireland
#School of Biotechnology, Dublin City University, Dublin, Ireland
¶Advanced Lung Disease and Lung Transplant Program, Mater Misericordiae University Hospital, Dublin, Ireland

WINNING ABSTRACT: Idiopathic Pulmonary Fibrosis (IPF) is a refractory and lethal interstitial lung disease characterized by loss of alveolar epithelial cells, fibroblast proliferation and extra-cellular matrix protein deposition. EBV, localised to alveolar epithelial cells of pulmonary fibrosis patients is associated with a poor prognosis.

In this study we utilised a microarray-based differential gene expression analysis strategy to identify molecular drivers of EBV associated with lung fibrosis. A549 cells and an alveolar epithelial cell line infected with EBV (VAAK) were used to identify genes whose expression was altered by EBV reactivation.

EBV reactivation by TGFbeta1 drives alterations in expression of non-canonical Wnt pathway mediators, implicating it in epithelial mesenchymal transition (EMT), the molecular event underpinning scar production in tissue fibrosis. Cell invasion, EMT correlated transcripts expression, GSK-3b and c-Jun activation were altered in response to non-canonical Wnt pathway regulation. The role of EBV in promoting fibrosis can be attenuated by antiviral strategies and inhibition of Wnt signalling.

Activation of non-canonical Wnt signalling pathway by EBV in epithelial cells suggests a novel mechanism of tissue fibrosis. These data present a framework for further description of the link between infectious agents and fibrosis, a significant disease burden.

Andrea P. Malizia
Advanced Lung Disease and Lung Transplant Programme, Genome Resource Unit, Mater Misericordiae University Hospital, Dublin, Ireland

MY JOB AND THE UNIT IN WHICH I WORK

I am a research fellow working as part of the Advanced Lung Disease and Lung Transplant Programme at Mater Misericordiae University Hospital, University College Dublin (Dublin, Ireland). From 2006, I have been funded by the National Heart and Lung Transplant Programme to work on the molecular mechanisms underlying the effect of Epstein–Barr virus (EBV) infection in idiopathic pulmonary fibrosis (IPF) development. I am currently supervised by Dr Jim Egan and Dr Peter Doran, and am pursuing my PhD degree at the Genome Resource Unit, a collaborative and interdepartmental research initiative at the University College Dublin, focusing on translational lung research.

MY WINNING POSTER AS PART OF MY RESEARCH

After gaining a BSc in 2004, I started to work in the Genome Resource Unit, University College Dublin. In 2006, I started a PhD programme with Dr Doran, studying the molecular aspects underpinning Herpesviridae infection in IPF, and their association with poor prognosis. A collaborative contract with Dr Egan as research fellow gave me the possibility to study the molecular aspect of IPF through a translational and clinical approach.

This joint collaboration produced important data about the key role of EBV in alveolar epithelial cell biology. In a collaborative study with Dr Dominic Keating, we identified genes whose expression were significantly altered in alveolar epithelial cells (A549) in response to transforming growth factor (TGF)-β1, interleukin (IL)-4 and -13 and EBV by using microarray and computational biology strategies. A pool of extracellular matrix (ECM) proteins or modulators of matrix turnover was curated *via* Onto-Compare and Gene-Ontology databases for baited cluster analysis of ECM associated genes. Of this grouping, members of the ADAM (A Disintegrin And Metalloproteinase domain-containing) family of genes were differentially expressed. ADAM gene expression was also identified in EBV infected A549 cells, as well as IL-13- and -4-stimulated cells, suggesting a potential role of EBV in ECM accumulation in IPF [1].

STATEMENT OF INTEREST: None declared.

To determine the role of host predisposition in IPF development, we utilised transgenic mice to simulate TGF-β1-related genetic predisposition to external stimuli, rather than a tissue-specific TGF-β1 transgenic model. We defined the fibrotic response in the transgenic animal population following epithelial cell injury, and characterised the transcriptomic changes in response to belomycin-induced injury. The double-injury mice were characterised by molecular patterns that are hallmarks of fibrosis, including ECM and cell growth, and regulation gene clusters. Further investigations will focus on characterising the combination of these factors in patients with IPF, with attention specifically paid to the role of the TGF-β1 gene as a prime for subsequent lung injury [2].

Based on contradictory findings of the role of TGF-β1 in EBV-infected and noninfected epithelial cells [3, 4], my main research project is based on investigating EBV lytic phase induction mechanism by TGF-β1 and chemical compounds to study molecular virus–host interactions in alveolar epithelial cells.

MY RESEARCH AS PART OF MY WORKING GROUP/ RESEARCH TEAM

The Translational Research Programme in Advanced Lung Disease, based at the Genome Resource Unit and Mater Misericordiae Hospital, is led by Dr Egan and Dr Doran, and includes Dr Keating and Dr Rauf Ahsan. Dr Egan is also a Consultant Respiratory Physician at the National Centre for Cardiothoracic Transplant. His research interests include chronic and acute lung diseases, with particular emphasis on interstitial lung diseases. Dr Doran is a lecturer at the University College Dublin, School of Medicine and Medical Science, and Director of the Genome Resource Unit. His research interests include the identification of the mechanisms of disease using gene discovery strategies. Furthermore, he has driven the creation of informatics solutions for the integration of clinical and biological data in human disease research. Dr Keating is a medical doctor who investigated IPF in pulmonary tissue samples *ex vivo* from transplanted lung. He had a major role in developing a stable, EBV-infected, alveolar epithelial cell line in collaboration with Dr Dermot Walls at the Dublin City University. Dr Ashsan is a surgeon who has collaborated in the study of IPF by using a primary human, small-airway, epithelial cell-based method to investigate their transcript expression associated with epithelial mesenchymal transition in response to TGF-β1.

THE IMPACT OF MY WORK ON CLINICAL OR RESEARCH PRACTICE

IPF is a common interstitial lung disease of unknown aetiology. Clinical deterioration in IPF is expected, and 5-yr survival ranges from 30–50%. Despite improvements in the diagnostic approach to IPF and active research in recent years, the molecular mechanisms of the disease remain poorly understood. This highly lethal lung disorder continues to pose major clinical challenges, since an effective therapeutic regimen has yet to be identified and developed.

Our research is supported by the observation that alveolar epithelial cell injury and repair is an important trigger event in the development of lung fibrosis. Using Epstein–Barr virus-infected alveolar epithelial cells, we have characterised the genomic alterations in these cells in association with an Epstein–Barr virus lytic phase status. The microarray approach we have described in the abstract uncovered the Wnt pathway as being a novel signalling in which transcripts are highly expressed in Epstein–Barr virus-infected cells in response to transforming growth factor-β1. The identification and characterisation of novel profibrotic mediators will have an impact on their use as possible therapeutic targets. Since no effective therapeutic options for patients with idiopathic pulmonary fibrosis exist at this moment, future work by our group and others will unravel the regulation of Epstein–Barr virus reactivation as a pro-fibrotic factor, and this may open up new therapeutic options for patients with idiopathic pulmonary fibrosis.

REFERENCES

1 Keating DT, Sadlier D, Patricelli A, *et al.* Microarray identifies ADAM family members as key responders to TGF-beta1 in alveolar epithelial cells. *Respir Res* 2006; 7: 114.
2 Haider Y, Malizia AP, Keating DT, *et al.* Host predisposition by endogenous transforming growth factor-beta1 overexpression promotes pulmonary fibrosis following bleomycin injury. *J Inflamm* 2007; 4: 18.
3 Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. *J Cell Biol* 1992; 119: 1017–1021.
4 Fukuda M, Ikuta K, Yanagihara K, *et al.* Effect of transforming growth factor-beta1 on the cell growth and Epstein–Barr virus reactivation in EBV-infected epithelial cell lines. *Virology* 2001; 288: 109–118.