Barts Cancer Institute

Barts and The London School of Medicine and Dentistry

Mechanostimulation of Integrin αvβ6 in Myoepithelial Cells Activates an Invasion Promoting Phenotype

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Pathological Society PhD Studentship: Summary Report

Introduction: For the majority of invasive breast cancers, progression follows transition through a preinvasive stage, ductal carcinoma *in situ* (DCIS) [1]. In DCIS, neoplastic cells proliferate within the lumen of the duct, and are restricted here by an intact myoepithelial cell layer which lies in contact with the basement membrane. At some point during progression, neoplastic cells breach the myoepithelial cell-basement membrane interface, and invade into the surrounding stroma, though the mechanisms underlying this transition are poorly understood (Figure 1A; top panel). With this, there is currently no robust way to determine which cases will and will not progress to invasion. An estimated half of DCIS cases will progress to invasion within a patient's lifetime [2], and concerns surround the overdiagnosis and overtreatment of DCIS [3]. Therefore, there is an urgent clinical need to identify prognostic markers to predict the progression of DCIS, in order to better direct therapeutic intervention [4]. Numerous studies have aimed to identify such markers that may predict the progression of DCIS, with most studies focusing on the comparison of neoplastic cells from DCIS with their invasive counterpart. These studies demonstrated no specific alterations associated with progression to invasion [5-9], and suggest DCIS is as genetically advanced as invasive breast cancer [10]. However, these early studies failed to incorporate the breast microenvironment, which comprises; the myoepithelial cell population and stromal compartment. Normal myoepithelial cells have been shown to exert a tumour suppressor function [11] in an autocrine and paracrine manner [12-15]. In DCIS, myoepithelial cells demonstrate an altered phenotype [16], and are suggested to switch to a tumour promoter function [17]. Our previous study showed that DCIS-myoepithelial cells exhibit the *de novo* expression of integrin $\alpha v\beta 6$, and this is associated with progression to invasion. *In vitro* studies found the expression of integrin $\alpha v\beta 6$ by myoepithelial cells promoted breast cancer cell invasion through TGFβ-mediated upregulation of MMP9 [18]. However, the mechanism regulating integrin $\alpha v\beta 6$ expression in DCIS-myoepithelial cells is unclear. Integrin $\alpha v\beta 6$ mediates TGFβ activation through the conformational modification of the latency associated peptide (LAP), which functions to maintain TGFβ inactive [19]. This mechanism of activation follows the localisation of the latent TGFβ complex into the extracellular matrix (ECM) through interactions between a latent TGFβ binding protein-1 (LTBP1) [20] and fibronectin (FN) [21]. In this manner, an integrin $\alpha v\beta 6$-positive contractile cell and a mechanically resistant FN matrix together provide the forces required to liberate active TGFβ from this complex [22]. The cancer-associated ECM demonstrates an increased stiffness [23]; accumulating experimental evidence demonstrates that this may be attributed to alterations in the deposition, composition and organisation of the ECM [24-25]. While the role of collagen in promoting stiffness in cancer has been well investigated [26-28], the role of FN and its alternatively spliced domains EDA and EDB; which is critical for the initial deposition of collagen in the ECM [29], is less clear.

Aim: To investigate the functional significance of up-regulated integrin $\alpha v\beta 6$ and FN expression by DCIS-MECs, in order to understand the mechanisms underlying the transition of DCIS to invasion. In doing so, we aim to generate a biomarker signature with which DCIS patients can be better stratified for appropriate management.

Materials and Methods: An immortalised myoepithelial cell line (1089) generated from normal breast was used to create normal (N-) 1089 cells [30]. These were then used to generate integrin $\beta 6$ overexpressing ($\beta 6$-1089) cells by retroviral transduction

of β6, to model DCIS-myoepithelial cells [18]. Using the Barts Cancer Institute (BCI)/Breast Cancer Now (BCN) tissue bank, primary myoepithelial cells were isolated from normal breast tissue. Together, with sophisticated mechanical modelling systems and classical molecular biology techniques, these cells were then used to investigate the function of alterations to the normal myoepithelial cell phenotype in the progression of DCIS.

Results: Progression of DCIS to invasion is accompanied by increased expression of integrin αvβ6 by myoepithelial cells and FN deposition. Confirming previous findings [18], no staining for integrin αvβ6 was seen in the adjacent normal tissue, whereas 45% of high-grade and 27% of non-high grade pure DCIS cases showed myoepithelial staining for integrin αvβ6, with a higher frequency of positivity in high-grade cases. The frequency of integrin αvβ6 expression by myoepithelial cells in DCIS with associated invasion is significantly higher than in pure DCIS ($p<0.05$) (Figure 1A; second panel; quantified in Figure 1B; top bar graph). Quantification of the amount of FN surrounding each duct demonstrated that the stromal region bordering DCIS lesions contained significantly more FN, and that increased further in DCIS with associated invasion (Figure 1A; third panel; quantified in Figure 1B; bottom bar graph). These findings reveal a progressive increase in both the expression of integrin αvβ6 by myoepithelial cells and the amount of FN in the stroma of breast cancer as a function of DCIS progression to invasion. To determine if there is a relationship between integrin αvβ6 and FN, we examined on a duct-by-duct bases the dual expression of integrin αvβ6 by myoepithelial cells and FN deposition surrounding the duct. We identified a significant association between the expression of both molecules ($p<0.0001$) (Figure 1C; quantified in Figure 1D). These findings suggest there likely exist a relationship between integrin αvβ6-positive myoepithelial cells and FN deposition surrounding the duct. Together, these data link DCIS-myoepithelial cells in altering the tumour microenvironment to facilitate DCIS progression to invasion.

FN expression is upregulated by integrin αvβ6-positive myoepithelial cells. To investigate myoepithelial cell expression of integrin αvβ6 in promoting the deposition of FN, we used established myoepithelial cell lines (b6-1089 and N-1089) with and without the expression of integrin αvβ6, respectively. Consistent with our tissue study, β6-1089, which model DCIS-MECs, exhibited higher levels of FN and FN-EDA expression at the protein (Figure 2A) and mRNA (Figure 2C) level compared to N-1089. In addition, CM obtained from β6-1089 demonstrate significantly higher levels of TFN and FN-EDA compared to N-1089 (Figure 2B). Moreover, β6-1089 organised FN into a fibrilar matrix (Figure 2D).

FN expression by integrin αvβ6-positive myoepithelial cells promotes TGFβ signalling pathways. Previous studies have demonstrated a role for a mechanically resistant FN matrix in liberating active TGFβ by integrin αvβ6 [22]. Allen and colleagues demonstrated the ability of β6-1089 to preferentially migrate and bind to LAP, and activate TGFβ compared to N-1089, and these functions are mediated exclusively by integrin αvβ6 [18]. Using the expression of phosphorylated SMAD2 as a marker of activate TGFβ signalling, we have shown that β6-1089 exhibit higher levels of TGFβ signalling at the basal level and in response to TGFβ stimulation, compared to N-1089, and this effect is abrogated using siRNA to integrin αvβ6 (data not shown). Loss of FN expression in β6-1089 by siRNA reduced cell migration ($p<0.01$) (Figure

3A) and adhesion ($p<0.01$) (Figure 3B) to LAP, as well as TGFβ signalling in response to TGFβ stimulation (Figure 3C). These data suggest FN facilitates integrin αvβ6 function to bind and migrate to LAP, as well as activating TGFβ signalling pathways.

FN expression by integrin αvβ6-positive myoepithelial cells promotes breast cancer cell invasion via MMP Secretion. We next investigated the role of FN in the tumour promoting function of integrin αvβ6-positive myoepithelial cells. Previous data demonstrated integrin αvβ6-positive myoepithelial cells promoted breast cancer cell invasion *in vitro* in a TGFβ-dependent upregulation of MMP9 [18]. Interestingly, we identified that the loss of FN expression led to the downregulation of breast cancer cell invasion *in vitro* (Figure 4A). Thirty-five proteases were measured in the conditioned media using a human protease array. We termed this protease signature the 'secretome'. We observed a downregulated secretion of the majority of proteases following knockdown of FN in β6-1089 (Figure 4B), in particular, those involved in promoting cancer cell invasion through degradation of the BM, including; MMP2 and MMP9. These findings were confirmed with qRT-PCR (Figure 4C) and gelatin zymography for MMP9 (Figure 4D). Similarly, we observed that these changes in the secretome were downregulated to levels seen in N-1089 or following knockdown of integrin αvβ6 expression in β6-1089 (data not shown). These data suggest that our model of DCIS-myoepithelial cells have a secretome that is regulated by the presence of both integrin αvβ6 and FN, which may promote breast cancer cell invasion.

Mechanical stretching of normal myoepithelial cells induces a DCIS phenotype associated with integrin αvβ6 expression. DCIS is characterised by the proliferation of neoplastic cells within the duct, which results in the expansion of the duct and as a consequence, stretching of the myoepithelial cell layer (Figure 5A). Analysis of DCIS duct size demonstrated that integrin αvβ6-positive DCIS ducts on average were larger than integrin αvβ6-negative DCIS ducts ($p<0.0001$; $145mm^2$ compared to $95mm^2$) (Figure 5B). Consistent with these findings, application of mechanical tension to normal myoepithelial cells; normal myoepithelial cell line (N-1089) and primary normal myoepithelial cells (N-1989 and N-1492), revealed an increase in integrin αvβ6 expression (Figure 5C, D and E). Moreover, mechanical stretching led to an upregulated secretome (Figure 6A, B and C), which represented that of β6-1089 (data not shown) and this secretome promoted breast cancer cell invasion *in vitro* ($p<0.01$) (Figure 6D). These results show an association between duct expansion and induction of integrin αvβ6 expression, and suggest evolving tissue mechanics during DCIS development activate the tumour promoting phenotype of DCIS-myoepithelial cells.

Conclusion: We provide the first study to assess the mechanoregulation of integrin αvβ6 in myopeithelial cells. We identified integrin αvβ6-positive DCIS ducts are larger than integrin αvβ6-negative DCIS ducts, and we demonstrate that mechanical stretching of normal myoepithelial cells, as seen in the expansion of breast ducts in DCIS, upregulates integrin αvβ6. Mechanoregulation of integrin αvβ6 alters the myoepithelial cell phenotype to an invasive promoting function. Furthermore, we show that integrin αvβ6-positive myoepithelial cells upregulate FN deposition, which facilitates MMP secretion that promotes breast cancer cell invasion *in vitro*. Moreover, expression of integrin αvβ6 by myoepithelial cells and periductal FN deposition is significantly associated with the progression of DCIS to invasion.

Personal Achievements

Awards

Sir Alastair Currie Poster Prize (1st) (July 2016)
Pathological Society of Great Britain and Ireland, Nottingham Pathology
'Investigating the Functional Significance of Aberrant αvβ6 and Fibronectin Expression in Myoepithelial Cells: Role in the Progression of DCIS'

PhD Day Best Poster Prize (June 2016)
Barts Cancer Institute, Queen Mary University of London
'Altered Microenvironment in the Progression of DCIS: The Role of the Myoepithelial Cell'

Travel Awards

Pathological Societry Meeting Bursary (£200) (May 2017)
Gordon Research Seminar Travel Bursary (£200) (February 2017)
Barts School Of Medicine And Dentistry Travel Grant (£200) (December 2016)
CRUK/BACR Student Travel Award (£1,000) (December 2016)

Publications

'Nelan R., **Hayward M**. and Jones JL. The Growth of Molecular Diagnostics: Stratified Medicine Programme, the 100,000 Genomes Project and the Future. Diagnostic Histopathology. 2017; 23(10):458-467

'Dreger S., Allen MD., **Hayward M**., Payne SJ., Reynolds L., Robinson S., Hodivala-Dilke K. and Jones JL. Myoepithelial Cells in Ductal Carcinoma In-Situ (DCIS) of the Breast Promote Angiogenesis Through TGFβ-Mediated Up-Regulation of MMP-9' (In Preparation)

'**Hayward M**., Allen MD., Gomm JJ., Knight MM., Marshall JF. and Jones JL. Mechanostimulation of Integrin αvβ6 in Myoepithelial Cells Activates an Invasion Promoting Phenotype' (In Preparation)

Invited Oral Presentations

Pathological Society of Great Britain and Ireland, Belfast Pathology – Belfast, UK (June 2016). 'Myoepithelial Cell Phenotype in DCIS Progression: Functional Significance of Integrin αvβ6 and Fibronectin'

Gordon Research Seminar: Fibronectin, Integrins and Related Molecules - California, USA (January 2017). 'Functional Significance of Integrin αvβ6 and Fibronectin in DCIS-Myoepithelial Cells: Role in Progression to Invasion'

Collaborative Experience

Collaboration with the Professor Martin Knight, at the School of Engineering and Materials Science, Queen Mary University of London

Future Prospects

Secured post-doctoral research associate position with Professor Valerie Weaver at the University of California, San Francisco

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