

Annexin A1 expression and intracellular location influence the invasive and metastatic capacity of lung adenocarcinoma

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Background: There is growing evidence to support an association between Annexin A1 (ANXA1) and poor prognosis in lung adenocarcinoma with increased levels of the protein found in both bronchoalveolar lavage fluid and serum. ANXA1 promotes an invasive phenotype in many cancer types, acting through both cognate formyl-peptide receptors and various intracellular pathways. Whilst published data suggest that increased overall ANXA1 expression contributes to tumourigenesis in lung adenocarcinoma there are no studies examining the association between ANXA1 subcellular localisation and tumour progression. We have observed that there is marked inter-tumour heterogeneity in overall ANXA1 expression and localisation within a small cohort of lung adenocarcinomas, highlighting the potential role for various mechanisms to be at play. This study aimed to evaluate whether ANXA1 expression and subcellular localisation changed in the progression from *in situ* (lepidic pattern) lung adenocarcinoma to invasive and metastatic disease and whether it was associated with epithelial-mesenchymal transition.

Methods: This study was approved by Lothian NRS Bioresource (study number: SR855). The study utilised surgical resection specimens from patients with primary lung adenocarcinoma in the Royal Infirmary of Edinburgh. Cases from 2015 and 2016 were identified using SNOMED-CT codes for lung adenocarcinoma. Overall ANXA1 expression and intracellular localisation (nuclear, cytoplasmic or membranous) were evaluated by immunohistochemistry with a scoring system based on percentage of cells stained and staining intensity as well as predominant distribution within the cell. Tumours were grouped as either lepidic pattern only (n=14), invasive with no nodal metastases (n=20) or metastatic carcinoma (n=20). Expression levels of E-cadherin and vimentin within tumour cells were used as markers of epithelial-mesenchymal transition.

Results: Total tumour cell ANXA1 expression was not associated with disease progression in our cohort. There was a trend towards increased expression of nuclear ANXA1 in metastatic tumours and invasive tumours compared to 'lepidic-only' tumours, although this was not statistically significant ($p = 0.301$) (Fig. 1). Nuclear ANXA1 expression was positively correlated with vimentin expression ($+0.328$, $p < 0.05$), and a negative correlation with E-cadherin expression was observed ($\rho = -0.107$), though not statistically significant ($p = 0.422$) (Fig. 2).

Conclusion: These pilot data suggest that there may be a role for nuclear ANXA1 subcellular localisation in driving invasion and metastasis in lung adenocarcinoma, potentially through upregulated epithelial-mesenchymal transition. This observation requires a larger cohort to be examined to determine whether consistent trends are seen. These data form part of a broader body of work with regards to ANXA1 in lung adenocarcinoma and will contribute to future funding applications.

These findings were presented as a poster presentation (*Nuclear Localisation of Annexin A1 May Promote an Invasive and Metastatic Phenotype in Lung Adenocarcinoma*; P19) at the Pathological Society meeting, **Maastricht Pathology 2018**

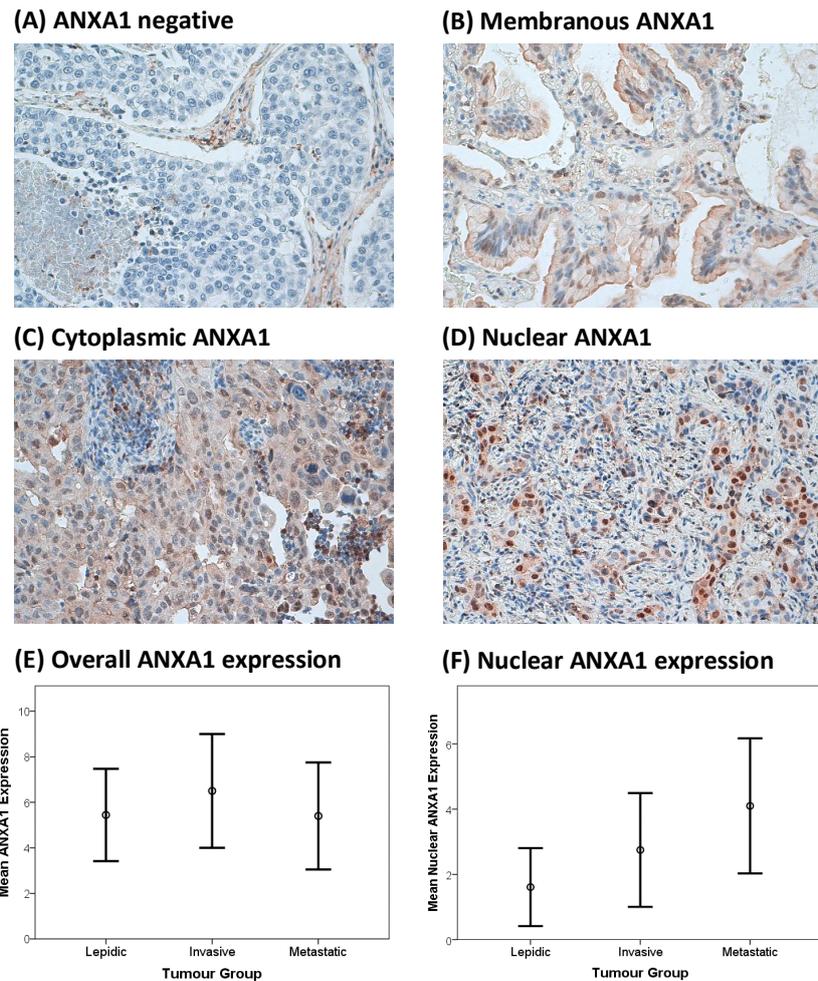


Figure 1. Annexin A1 expression in lung adenocarcinoma. (A-D) Examples of differences in subcellular localisation of ANXA1. Negative staining in an acinar adenocarcinoma (A). Membranous staining, intensity score 3, in a lepidic tumour (B). Cytoplasmic staining, intensity score 2, in a lymph node metastatic deposit (C). Nuclear staining, intensity score 3, in a lymph node metastatic deposit (D). Original magnification: 200x. (E) No significant difference in overall ANXA1 expression was seen between groups was identified ($p = 0.723$; Kruskal-Wallis test). (F) Whilst an upwards trend was noted from lepidic to invasive to metastatic tumours, this was not significant ($p = 0.301$; Jonckheere-Terpstra test). Error bars represent the 95% confidence interval.

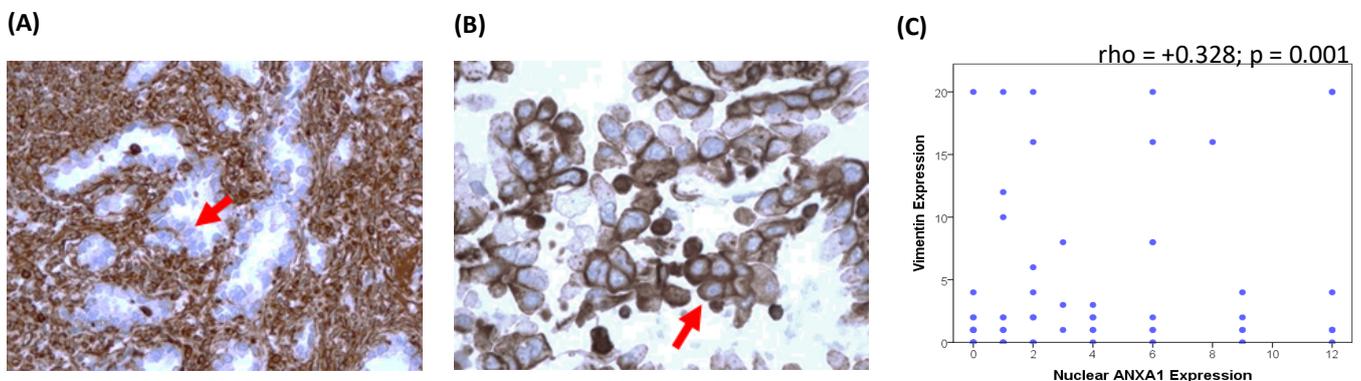


Figure 2. Epithelial-mesenchymal transformation in relation to nuclear ANXA1 expression. (A, B) Variation in vimentin expression between different lung adenocarcinomas (red arrows highlight tumour cells) with a strong correlation observed between vimentin and nuclear ANXA1 expression (C).