Pathological Society Pump Priming small grant: Prof Emad A Rakha:

Grant Reference No: 1158.

**Leukocyte associated immunoglobulin like receptor-1 confers poor prognosis in Invasive Breast Carcinoma: Transcriptomic driven study**

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**BACKGROUND:**

Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1/CD305) is a transmembrane glycoprotein and a member of immunoglobulin super family expressed on most immune cells. LAIR-1 is an inhibitory receptor involved in down-regulation of immune response and cell differentiation [1-3]. The immune system plays an important role in breast cancer (BC) behaviour and outcome. Tumour-inﬁltrating lymphocytes (TILs) are associated with outcome in BC and their existence before chemotherapy is a good phenomenon [4] but lymphocytes secrete IL-6 and IL8, which in turn activate PI3K/AKT, STAT3 signalling, and generate a positive feedback loop between the tumour cells and immune microenvironment. The majority of TILs with prominent CD8+ T cells are linked with a better prognosis [5] whilst Foxp3+ or PD-1+ T cells inﬁltration mediates worse prognosis [6]. In addition, multiple transmembrane and extracellular matrix collagens, which are high affinity ligands for LAIR-1 [7], have been implicated as the avenues for metastasis in BC [8]. The expression of LAIR-1 in both tumour cells and the immune cells indicates a regulatory role of LAIR-1 in tumour biology and requires further studies however; the biological role of LAIR-1 in BC has yet to be elucidated.

We evaluated the *LAIR-1* mRNA in the large METABRIC BC cohort (n=2000)[9]. This revealed that up-regulation of *LAIR-1* mRNA is positively associated with higher histological grade (p<0.00001), Nottingham Prognostic Index poor prognostic group (p<0.0001), medullary-like tumours (p=0.024), and tumours with triple negative phenotype (p<0.05). High expression levels of *LAIR-1* mRNA were significantly associated with METABRIC Integrative Clusters with upregulation within intClust.10 (p=<0.0001) representing patients with poor outcome and basal-like PAM50 intrinsic subtype. Univariate survival analysis demonstrated poor outcome for BC patients showing high expression of *LAIR-1* mRNA (p=0.026).

From the aforementioned studies we hypothesised that LAIR-1 expression is associated with poor prognosis in BC. Thus, in this study we investigated the association between LAIR-1, clinicopathological factors and patient outcome at protein level in a well characterised early-stage BC cohort. I addition the, relationship between LAIR-1 protein expression with total and subtype (B and T cell and histiocytic markers [10, 11] were also investigated.

**METHODS:**

Specificity of LAIR-1 antibody was validated by western blotting prior to immunohistochemistry (IHC). Full-face BC tissue sections and TMAs were immunoassayed using Novolink Max Polymer Detection system (Leica, Newcastle, UK) as previously described[12]. Immunoreactivity of LAIR-1 cytoplasmic expression in invasive tumour cells were individually assessed using the histochemical score (H-score) and its association with clinico-pathological paraments and patient outcome were evaluated.

Differential expression of LAIR-1 was evaluated in different molecular subtypes of BC including luminal A (MCF-7, T47D) and B (ZR-75-1, MDA-MB-175), HER2 positive (SKBR-3) and Triple Negative (MDA-MB-231 and MDA-MB-468) cell lines. Based on the screening of LAIR-1 expression in BC cell lines, highest expression was observed in SKBR-3 and MDA-MB 231. LAIR-1 were transfected with Silencer Select Pre-Designed functional siRNAs and a scrambled negative control and the efficiency of gene silencing were assessed using Western Blotting to ensure significant levels of knock down (KD). The effect of LAIR-1 KD on proliferation was assessed by the 3–(4,5–dimethylthiazol‐2–yl) ‐2,5–diphenyltetrazolium (MTT) assay. Cell migration was determined using the Radius™ 24-well from Cell Biolabs (CBA-125; San Diego, CA).

**RESULTS:**

Full-face BC tissue sections were used to evaluate the pattern of LAIR-1 protein expression prior to staining of TMAs. This showed uniformly weak LAIR-1 expression in normal glandular epithelium and DCIS. On TMAs, a variable degree of LAIR-1 protein expression in invasive BC was observed. Out of 569 informative TMA cores, 49.4% showed negative/low expression (Fig. 1a) in the cytoplasm while 50.6% showed high expression (Fig. 1b).

High LAIR-1 cytoplasmic expressions were associated with high tumour grade (p<0.0001), poor Nottingham Prognostic Index (NPI; p=0.012), hormone receptor negativity (p<0.01), among IHC subtypes associated with triple negative and human epidermal growth factor 2 (HER2) + tumours (p=0.019). High LAIR-1 expression positively associated with Cyclin B1(p=0.010) and signalling pathway associated markers; epidermal growth factor receptor (EGFR; p=0.045). Elevated expression of LAIR-1 was also associated with T-cell markers (CD3, CD8, and FOXP3; p<0.01) and histiocytic marker (CD68; p<0.001). Breast cancer-specific survival (BCSS) of patients with tumours expressing high cytoplasmic LAIR-1 was significantly shorter than that of the negative/low expression subgroup (p=0.002). Multivariate analysis revealed that increased LAIR-1 protein is an independent risk factor for shorter BCSS (p=0.039; Fig. 1c).

We conﬁrmed that SiRNA targeting LAIR-1 reduced the level of endogenous LAIR-1 in BC cells compared to control cells. LAIR-1 transfected BC cells showed significant reduction in the cell proliferation and migration activity.

**CONCLUSIONS:**

This study provides evidence for the prognostic value of LAIR-1 in invasive BC. Strong positive association with immune cell markers and LAIR-1 warrant further studies to assess them individually and in combination along with the immune check point proteins.

**Outputs from the study in which the Pathological Society has been acknowledged:**

We presented part of this work at Leeds Pathology July 2019 Conference, Harrogate, UK.

We are currently working on a manuscript publishing these data and the society will be fully acknowledge and informed when this goes to press.



**Figure 1:** **immunohistochemical expression of LAIR-1 in BC:** showing low immunoreactivity (A) and high immunoreactivity on TMAs (B); images are at x40 magnification. (C) showing the forest plot for multivariate analysis for breast cancer-specific survival.

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