LRRC15: a Regulator of Immune Function in the Tumour Microenvironment

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Introduction

- LRRC15 is a cell-surface protein which has been identified as a potential biomarker for cancer prognosis.
- It is upregulated in various solid tumours and very sparingly expressed in healthy tissue - this makes it a good target for prospective cancer therapies.
- Within healthy tissue, it is almost exclusively expressed in immune barriers, implying there could be a link between LRRC15 and the behaviour of immune cells.
- It seems to be upregulated in metastatic tumours, suggesting it might impact the likelihood of tumour metastasis.

Null Hypothesis: There is no correlation between LRRC15 expression and the occurrence of myeloid-derived suppressor cells, M2 macrophages and M1 macrophages within the tumour microenvironment.

Methodology

- Multiplexed immunofluorescence using a Leica Bond RX autostainer
  - A triplicate set of 44 tissue cores was obtained from a tissue microarray recipient block.
  - CD11b, CD86 and LRRC15 receptors were labelled with primary antibodies.
  - These were visualised with Tyramide signal amplification.
  - Hoechst (DAPI) counterstaining was used.
- Image Acquisition of fluorescence images using a Zeiss Axio Scan Z1 Digital Slide Scanner
  - A fluorescence profile was used to produce four fluorescence images.
  - The images were superimposed to make one multiplex immunofluorescence image with four channels.
- Immunohistochemistry
  - CD206 and S100 receptors were labelled, then visualised with a polymer conjugated with HRP and with Green chromogen, respectively.
  - Haematoxylin counterstain was then used.
- Image acquisition of brightfield images using a Zeiss Axio Scan Z1 Digital Slide Scanner
  - A brightfield profile was used to produce two brightfield images.
- HALO AI™ image analysis
  - Brightfield images were deconvolved.
  - These images were registered and fused with the multiplex immunofluorescence image.

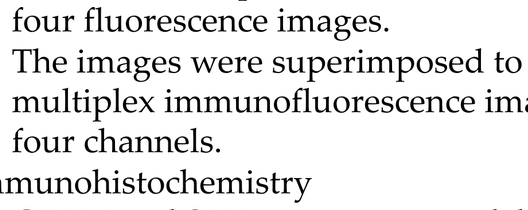


Fig. 1. Image Fusion

  - RF-Tissue VS Background classifier was created to distinguish tissue from background.
  - Two more classifiers where created, one to distinguish S100-positive tissue from S100-negative, the other to identify areas of LRRC15 positivity.

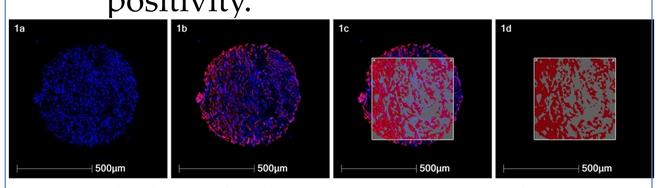
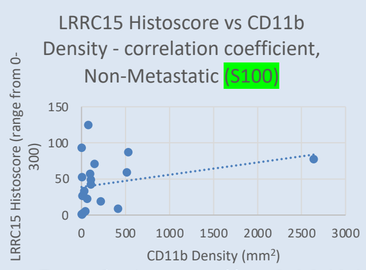


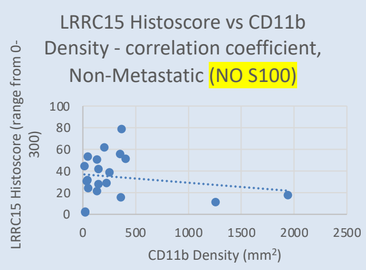
Figure 2. Real-time tuning being used to create the RF-LRRC15 VS NO-LRRC15 classifier

  - A default classifier was used to distinguish individual nuclei.
  - Batch processing was undertaken by HALO AI to quantify the expression of CD206 (M2 macrophages), CD86 (M1 macrophages) and CD11b (myeloid-derived suppressor cells)
- Data collation
  - Two-tailed Mann Whitney tests were completed in Excel.
  - Correlation coefficients were calculated between each immune cell and LRRC15 and their significance was ascertained using two-tailed T tests.

Results



Correlation coeff.: +0.286063
P-value: 0.915832



Correlation coeff.: -0.180947
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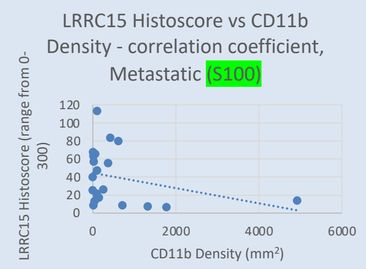

Correlation coeff.: -0.305826
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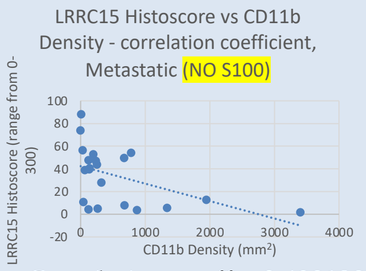

Correlation coeff.: -0.498138
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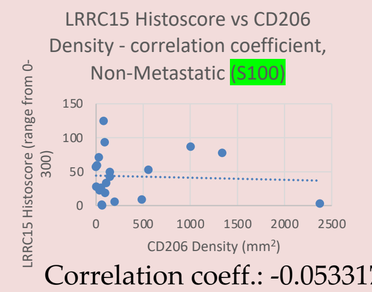

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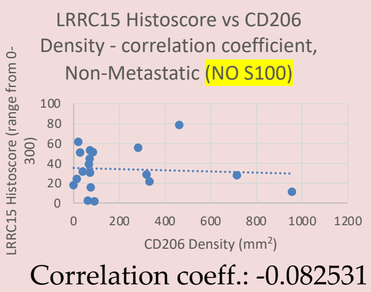

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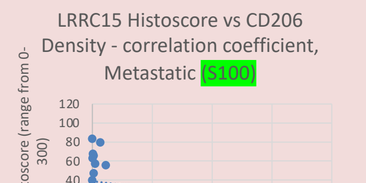

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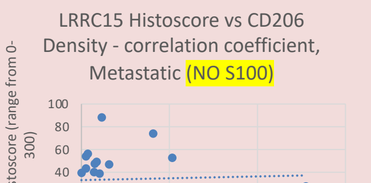

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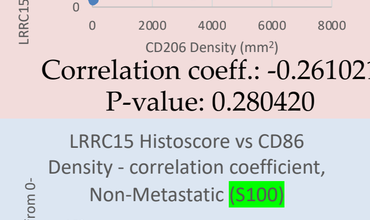

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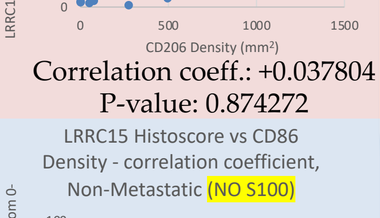

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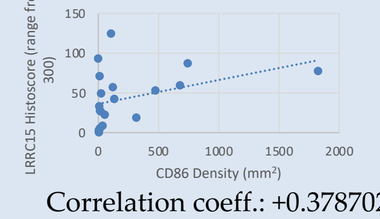

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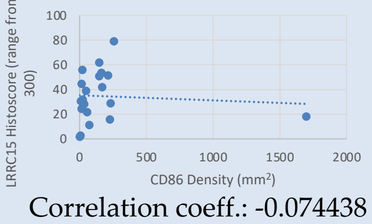

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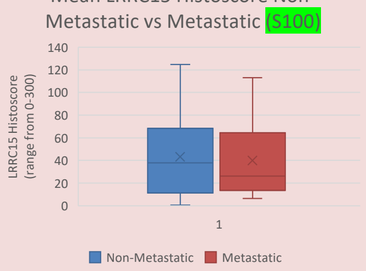

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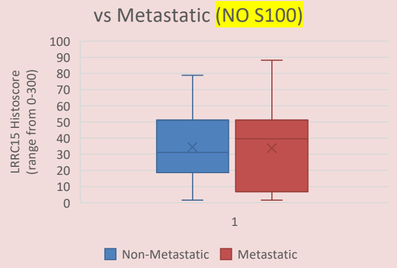

P-value: 0.86502

Discussion

- No meaningful correlations between the expression of any of the innate immune cells and the presence of LRRC15 were found.
- What are some ulterior reasons which may have caused this?
  - Very small data set
  - High potential for error during the image analysis stage, for example: creation of the RF-Tissue vs Background classifier, quality controlling of the tissue cores and creation of the LRRC15 vs NO LRRC15 classifier
  - Loss of tissue during quality controlling causes small data set to shrink further
- Advantages of AI-assisted oncological diagnosis:
  - Added efficiency and precision
  - Gives potential to use past patient data to create algorithms which can use biomarkers to precisely predict prognosis and to ascertain which therapies would be most effective – this gives potential to create treatment plans more personalized to each patient and to improve patient outcomes.
- Risks of relying on AI-assisted oncological diagnosis:
  - It is only as good as the human who creates the algorithms and should be used with this in mind so that diagnoses aren't missed.
- Although there were no correlations, possible trends were seen.
  - Among cancer cells (S100+ve), **less** LRRC15 was noted in metastatic cancer than in non-metastatic cancer. Within stroma (S100-ve), **more** LRRC15 was noted in metastatic cancer than in non-metastatic cancer.
    - Why? Possibly due to increased TGF-β exposure, as it's produced in the stroma and increases LRRC15 expression.
    - Significance? Implicates LRRC15 as a potential stromal biomarker for more developed cancer
  - BUT this is all speculation as the results aren't conclusive.
  - More MDSCs, M1 macrophages and M2 macrophages were seen in metastatic cancers than in non-metastatic cancers within both S100-ve and S100+ve tissue.
    - Significance? This could implicate all three of these immune cells as being tumorigenic – however, inconclusive results mean this cannot be confirmed.
- What does this mean for LRRC15?
  - We cannot conclusively confirm or refute whether it regulated the immune system or is a biomarker for worse prognosis.

Conclusion

Despite some expected and some unexpected trends between LRRC15 expression and the density of myeloid-derived suppressor cells, M2 macrophages and M1 macrophages, there were no statistically significant links between them. Therefore, we cannot confidently reject our null hypothesis, but with more extensive research this might be possible in the future. Further study is needed to understand LRRC15's precise role in regulating the innate immune system.

Acknowledgements

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References

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2) Purcell JW, Tanlimco SG, Hickson J, Fox M, Sho M, Durkin L, Uziel T, Powers R, Foster K, McGonigal T, Kumar S, Samayoa J, Zhang D, Palma JP, Mishra S, Hollenbaugh D, Gish K, Morgan-Lappe SE, Hsi ED, Chao DT. LRRC15 Is a Novel Mesenchymal Protein and Stromal Target for Antibody-Drug Conjugates. Cancer Res. 2018 Jul 15;78(14):4059-4072. doi: 10.1158/0008-5472.CAN-18-0327. Epub 2018 May 15. PMID: 29764866.
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