Cell Communication in the Immune System

Profiling Notch ligand expression in human tissues

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Introduction

- Dendritic cells (DCs) are a type of white blood cell that activate and control the response generated by other white blood cells to threats e.g. viruses.

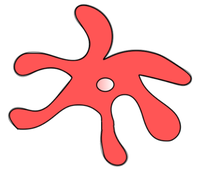


Figure 1: Dendritic cell

- They present foreign substances called antigens to CD8+ T cells, which kill infected cells. This is particularly important in the response against viruses and tumours.
- Several subtypes of DC exist; the rarest are classical type 1 DCs (cDC1s), which have an enhanced ability to present antigen to CD8+ T cells.
- All white blood cells originate in the bone marrow as stem cells. These divide into progenitor cells.
- The progenitor cells receive signals at certain stages that determine their change into specific cell types and subtypes.
- Signalling between different cells occurs via receptors and ligands (molecules that activate receptors) on their surface.
- Recent work has determined that a signalling system called Notch is important for the transformation of progenitor cells into increased numbers of cDC1s[1].
- Because of the enhanced ability of cDC1 to activate CD8+ T cells, they have the potential to be used therapeutically, e.g. in anti-tumour vaccines.
- Notch signalling could be used to increase how many cDC1s are produced. However, it is not currently understood in which body tissues the signal is available.

Aim

This study aims to determine whether Notch ligands are located in human bone marrow, spleen or skin and to what extent by quantifying the DNA of the different ligand and receptor types.

Methods

- Cells were extracted from human skin, spleen and bone marrow samples (three of each) and frozen until needed.
- Notch receptors are found on cells that carry a protein called CD45, whilst Notch ligands are found on cells which do not have CD45.
- Cells were therefore sorted via presence or absence of CD45 using fluorescence-activated cell sorting into positive and negative samples.

Methods

RNA Extraction

- Cell samples were defrosted and the RNA extracted.
- DNA was also removed to prevent contamination.
- A spectrophotometer was used to determine the purity of the RNA by measuring the light emitted.

Copy DNA Synthesis

- The RNA extracted was then used as a template to produce copies of DNA that were complementary to it.
- This was necessary for the next step.

Quantitative Polymerase Chain Reaction (QPCR)

- QPCR is used to measure the amount of DNA produced over time.
- A dye is used that binds DNA and fluoresces.
- Therefore, more DNA results in a higher fluorescence.

Results

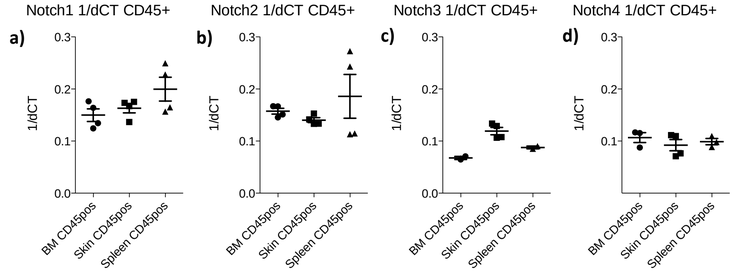


Figure 2[2] – Expression (presence) of the receptors in different tissues
Notch 1 - 4 are the receptors found on white blood cells, which are positive for CD45. Notch 1 and 2 (figures a and b) were more highly expressed than Notch 3 and 4 (figures c and d), particularly in the spleen.

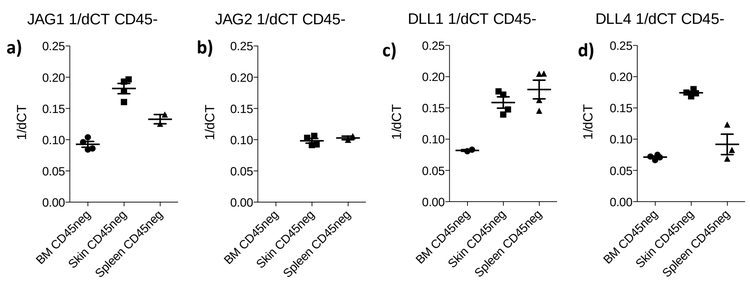


Figure 3[2] – Expression of the ligands in different tissues
Ligands Jagged 1 and 2, (JAG1,JAG2) Delta-like ligands (DLL) 1 and 4 are found in CD45 negative tissue cells. DLL1 was expressed highest in the spleen (figure c), with similar levels to DLL4 in skin (figure d). JAG1 was expressed highly in skin (a). All ligands were expressed in low levels in bone marrow, where JAG2 was not expressed at all (b).

Results

Figures 4a, b & c – the expression of all ligands and receptors in the skin (4a), bone marrow (4b) and spleen (4c) found via QPCR. Two samples of each tissue type were used. The delta CT value shows the fold change when compared to the CT value of the control (GAPDH, not shown). Therefore, a lower value represents higher levels of expression.

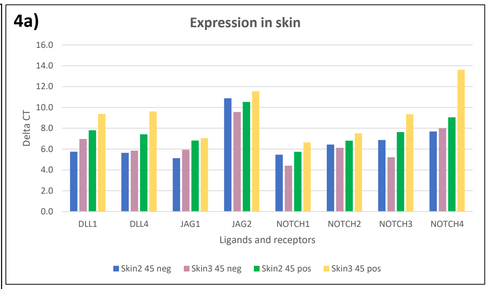
DLL1 was expressed highly in the skin and CD45 negative spleen samples, with little expression in bone marrow.

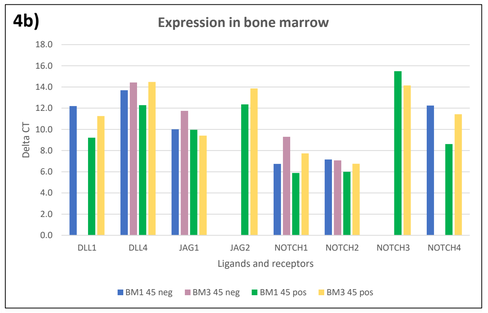
DLL4 and JAG1 were also expressed highly in skin, with little expression elsewhere.

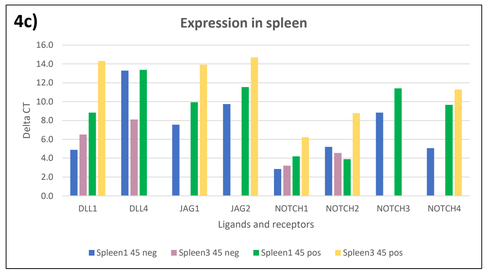
JAG2 was expressed at low levels in skin and spleen, with no expression in CD45 negative bone marrow.

Notch 1 and 2 were expressed highly at similar levels across all samples. Notch 3 and 4 were mostly expressed in the skin at lower levels compared to Notch 1 and 2, with low expression elsewhere.

Notch 3 was absent in both CD45 negative bone marrow samples. It was also not expressed in the cells from the third spleen sample.







Conclusions

- Overall, Notch ligands were found in the highest amounts in the skin and lowest in the bone marrow.
- Notch 1 and 2 were found at similar levels in all the tissue types. This suggests notch signaling is determined more by the presence of ligands than receptors.
- Large number of cDC1s are found in the spleen. DLL1 is the most important ligand for production of cDC1s; therefore it is found at its highest levels in the spleen. DLL1 was at its lowest in the bone marrow where cDC1s are present in the lowest amounts.
- Previous work has identified that Notch 2 and DLL1 are most important for cDC1 production; the results of this study support that[1].

Acknowledgments

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References: 1. Kirkling et al. Notch Signalling Facilitates In Vitro Generation of Cross-Presenting Classical Dendritic Cells. Cell Reports. 2018;23:3658–3672
2. Figures 2 & 3: Venetia Bigley.