Conventional type 1 dendritic cells and natural killer cells demonstrate strong correlation to cytotoxic T lymphocyte infiltration in cervical cancer tumors

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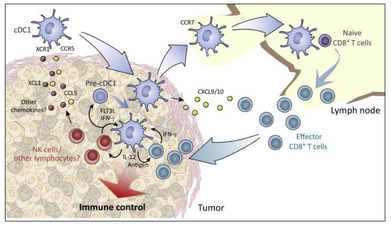
Introduction

The ability of T cells to mediate anti-tumor immunity has been harnessed to develop some of the most successful immunotherapies in recent years. Although direct presentation of tumor antigens by tumor cells plays an important role in the effector function of cytotoxic T lymphocytes (CTLs), cross-presentation by professional antigen presenting cells such as dendritic cells (DCs) is vital for priming naive CD8+ T cells and developing a sustainable cytotoxic response. Natural killer (NK) cells within the tumor microenvironment (TME) recruit a specific population of DCs called conventional type 1 DCs (cDC1s) into the TME by secreting chemokines such as CCL5 and XCL1. However, these cells are very low in abundance and are characterized by the expression of numerous markers, making their detection in the tissue context challenging.

Therefore, to interrogate the presence of cDC1 and NK cells in the TME and reveal their spatial relationship to each other we utilized the highly sensitive and specific RNAscope Multiplex Fluorescence *in situ* hybridization (ISH) assay.

Conventional dendritic cells 1 (cDC1) potentiate anti-tumor immunity



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- cDC1 cells get recruited at the site of the tumor by chemokines such as XCL1 and CCL5 secreted by NK cells.
- The cDC1 cells take up tumor antigens and present it to the naïve CD8+ T cells in the tumor draining lymph node.
- Additionally, chemokines secreted by cDC1 cells within the TME can recruit CD8+ effector T cells and re-stimulate them to potentiate the anti-tumor immune response.

Methods

- **Objective:** To identify conventional dendritic cells and other immune cells associated with recruiting cDC1s to the tumor microenvironment using target-specific probes and RNAscope Multiplex Fluorescent Assay V2
- **Sample used::** 4 FFPE Cervical cancer tumor samples
- **Assay:** RNAscope Multiplex Fluorescent Assay V2 to visualize 4 targets simultaneously on FFPE tissues
- **Quantification:** ImageJ software was used to count the total number of cells in a field of view based on DAPI staining . cDC1 , NK and T cells were counted manually.

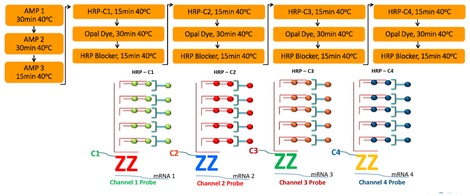
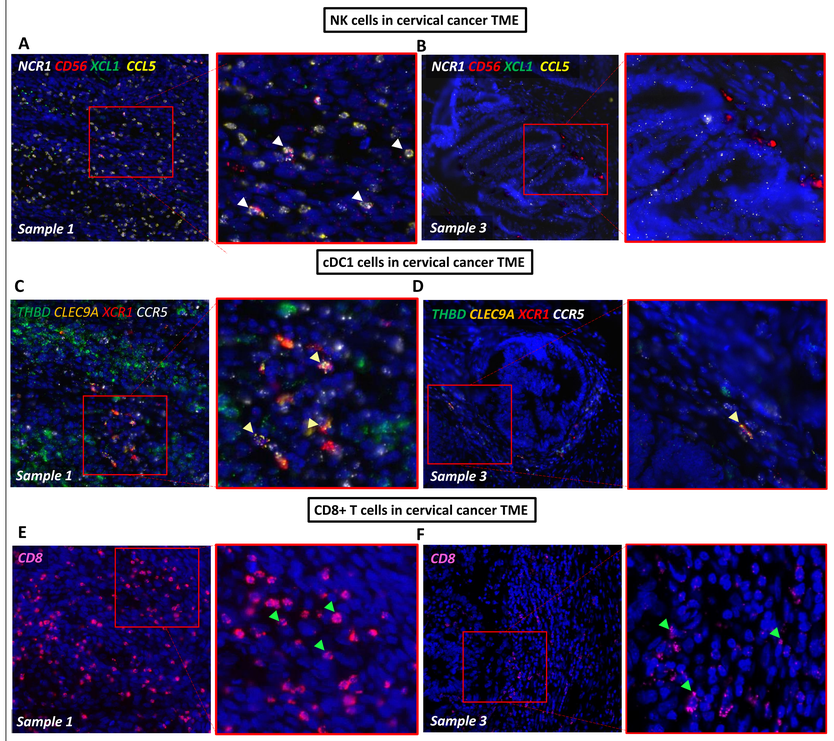


Figure 1: RNAscope Multiplex Fluorescent V2 assay workflow schematic

Results

RNAscope demonstrates co-localization of cDC1, NK and T cells in cervical cancer tumor niches



Percent positive cells across 4 different cervical cancer tumors

Cell types	Sample 1	Sample 2	Sample 3	Sample 4
NK cells	1.3	1	0.2	0.9
cDC1 cells	1.3	2.1	0.7	1
CD8+ T cells	19.5	15.5	12.5	15.2

Graphical representation of cell number quantification

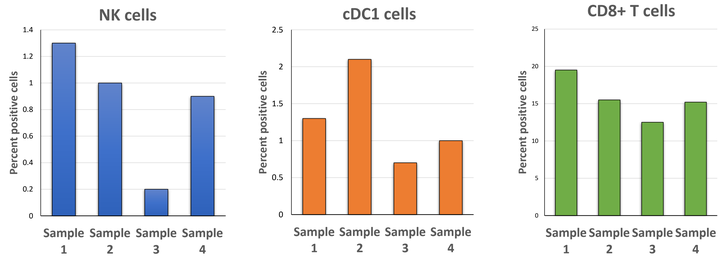


Figure 2: Detection of cDC1, NK and T cells in cervical cancer tumors using the RNAscope Multiplex Fluorescent V2 assay: A, using a combination of specific marker probes, NK cells (white arrows) were detected in cervical cancer sample 1. B, cervical cancer sample 3 demonstrated significantly lower levels of NK cells. C, Similarly, using specific marker probe combination, a sub-population of DC cells, cDC1 (yellow arrows) were identified in the same tumor niche as NK cells in sample 1, whereas, D, sample 3 showed very few cDC1 cells in the tumor. E, CD8+ T cells were detected in sample 1 to reveal high levels of CD8+ T cells (green arrows) in the same tumor niche as NK and DC cells. F, sample 3 demonstrated lower expression of CD8 indicating lower CD8+ T cell infiltration in the tumor niche.

Summary

- Our results revealed a strong correlation between the presence of NK cells and cDC1 cells within 3 out of 4 cervical cancer samples.
- The NK cells showed expression of the chemokines *XCL1* and *CCL5,* which are the ligands for XCR1 and CCR5 receptors respectively, that are expressed on cDC1 cell surface.
- The *XCR1*$^{+}$/*CCR5*$^{+}$ cDC1 cells may have been potentially recruited by chemokine secreting NK cells.
- Samples with high cDC1 and NK cells also showed significantly higher levels of CTL recruitment, as indicated by the presence of *CD8*$^{+}$ T cells.
- One of the 4 cervical cancer samples demonstrated relatively lower levels of NK cells which correlated with lower cDC1 cells and in turn lower CTL infiltration.

Conclusion

By utilizing the RNAscope Multiplex ISH assay we were able to identify and visualize the spatial relationship between NK cells, CTLs, and cDC1 cells, a rare subset of DC cells that excel at presenting tumor antigens to T cells. Using this technology, it is possible to spatially interrogate the TME and detect specialized immune cells when assessing response to immunotherapies.

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