534 BETTER IMMUNE PROFILES ON ELDERLY COLORECTAL CANCER PATIENTS CORRELATED WITH 1 YEAR DISEASES FREE SURVIVAL (DFS)

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Background Colorectal cancer is the most common gastrointestinal tract cancer, there are many factors which plays an important role on short and long term outcome prior to surgery and adjuvant therapy. For many decades, oncological factor has been state as the main of favorable outcome which could be evaluated by diseases free survival (DFS). Current study already evaluated the immune factor which has an important role on the progression on this colorectal cancer patients.
Methods We evaluated the colorectal cancer patients whose has been diagnosed as adenocarsinoma colon and rectal from operative speciments. The blood level of CRP, IL-6, IFN?, CB-8, IG G and IG M will be examined initially before the operative procedure done. All patients were stage III colorectal adeno carcinoma and adjuvant chemotherapy has been administered for six months period. The patients whose could not completed the adjuvant chemotherapy will be excluded from the study. The outcome of this study will be evaluated the 1 year disease free survival based on the abdominal CT Scan and chest x-rays.
Results There were 2 groups on this study, adults (< 60 years old) and elderly (>60 years old). 62 patients were included, 30 adults patients and 32 elderly patients has been evaluated for the immune profiles. We found the signifance difference were on the level of CRP, IL-6,IFN?, CB-8, IG G and IG M ($p < 0.05$). All patients had R0 resection and completed the adjuvant chemotherapy. 5 patients in the adult colorectal cancer group has locoregional and distant metastases in the lung and liver after 1 year evaluation. On the contraty, we could achieved 1 year diseases free survival in the elderly patients ($p < 0.05$) respectively.
Conclusions Elderly colorectal cancer patients has better immune profiles and has better 1 year disease free survival.
Ethics Approval The study has approved by Ethical Committe of Health Study Faculty of Medicine Sebelas Maret University, Indonesia. Approval number: 21457/BD/2020
Consent All of the patients already have a consent for this study

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535 ELECTROPORATION OF B CELLS IS CORRELATED WITH CELL SIZE CHANGE DURING B CELL EXPANSION

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Background Primary B cells are an important target for investigation and transfection of B cells is considered difficult. Electroporation is a very useful technology for transfection but its application on B cells has been unsatisfactory with low efficiency and low viability. The first reason is the small size of B cells compared to cell lines and the second reason is the low abundance of B cells in human PBMC. Since we had previous exprience with T cell electroporation, we sought to extend our knowledge on electroporation to B cells.
Methods Here we studied the B cell electroporation in PBMC samples and found that it is preferrable to electroporate the B cells in the PBMC mixture and B cells can be purified after electroporation if necessary. In this fashion, the total cell number in electroporation is boosted by other cell types in the PBMC and it helps B cell electroporation. Furthermore, we studied expanded B cells and found that they have a larger size than unstimulated B cells and the size increase is correlated to a decrease in electroporation voltage, consistent with the electroporation principle that larger cells need a lower voltage.
Results When B cells are expanded, the electroporation efficiency is similar to common cell lines and it becomes easy to do gene expression or genomic modification.
Conclusions Our studies elucidated the mechanism of difference between unstimulated B cells and expanded B cells and could be useful in helping the research on B cells.

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536 DIVERGENT CANCER ETIOLOGIES DRIVE DISTINCT B CELL SIGNATURES AND TERTIARY LYMPHOID STRUCTURES IN HEAD AND NECK CANCER

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Background Current FDA-approved immunotherapies aim to reinvigorate CD8+ T cells, but the contribution of the humoral arm of the immune response in human cancer remains poorly understood. B cells within tissues can mediate anti-tumor immunity and regulate immune responses by presenting antigen and producing tumor-specific antibodies and immunomodulatory cytokines. Head and neck squamous cell carcinoma (HNSCC) can be induced by human papillomavirus (HPV+) and carcinogens such as tobacco and alcohol (HPV-), and the immune infiltrate is quite distinct in the two etiologies, in particular, increased B cells in HPV+ HNSCC patients. Further, increased B cells in HNSCC patients correlate with improved patient survival. Our study seeks to differentiate B cell phenotype, function and location in HPV+ and HPV- HNSCC to identify putative B cell-centric immunotherapeutic targets.
Methods We utilized a multi-level approach to clearly categorize B cells in HNSCC patients. Single cell RNA sequencing (scRNAseq) was performed on CD45+ tumor infiltrating lymphocytes (TIL) from HPV+ and HPV- HNSCC patients. HNSCC TIL and PBL were stained via spectral cytometry (Cytek Aurora,25 parameters) for unbiased analysis of B cell subsets via computational spectral unmixing. Paraffin embedded slides from HNSCC primary tumors were utilized for multispectral immunofluorescence (mIF) to identify tertiary lymphoid structures (TLS) and identify differences in HPV+ and HPV- disease.
Results We demonstrated distinct trajectories for B cells in HPV+ and HPV- disease. HPV- HNSCC tumors mainly contained memory B cells and plasma cells, while the B cells in HPV+ HNSCC were naïve and germinal center (GC). Further, we quantified B cells and CD4+ T cells in TLS, and germinal center-like TLS were associated with improved outcome in HPV+ disease. We also observed that transcriptional and protein expression of Semaphorin A (SEMA4a) was restricted to GC B cells and increased on GC B cells in HNSCC patients compared to healthy tonsils. Additionally, we identified distinct

waves of gene expression in GC B cells in HNSCC tumors, ultimately revealing a novel transitional state for GC B cells in the tumor microenvironment (TME).

Conclusions Understanding B cell function in human cancers and how different TMEs influence B cells and TLS are important for devising novel therapeutic options for cancer patients. Ultimately, development of therapeutics to enhance B cell responses in the TME should be prioritized as a compliment to T-cell mediated therapies.

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537 CONVENTIONAL TYPE 1 DENDRITIC CELLS AND NATURAL KILLER CELLS DEMONSTRATE STRONG CORRELATION TO T LYMPHOCYTE INFILTRATION IN CERVICAL CANCER TUMORS

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Background The ability of T cells to mediate anti-tumor immunity has been harnessed to develop 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.

Methods Therefore, to interrogate the presence of cDC1 and NK cells in the TME and reveal their spatial relationship we utilized the highly sensitive and specific RNAscope Multiplex Fluorescence in situ hybridization (ISH) assay. Probes for XCR1, THBD, CLEC9A, and CCR5 were used to identify cDC1 cells within 4 cervical cancer tumors. These tumors were then assessed for the presence of NK cells by using specific marker probes such as CD56 and NCR1 and chemokines XCL1 and CCL5. Finally, CTLs were visualized to determine if there is a correlation between the presence of cDC1 and NK cells and CTL infiltration within the cervical cancer tumors.

Results Our results revealed a strong correlation between the presence of NK cells, cDC1 cells, and CTLs 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 respectively, suggesting that the XCR1+/CCR5+ cDC1 cells may have been potentially recruited by these NK cells. Regions high in cDC1 and NK cells also showed significantly higher levels of CTL recruitment, as indicated by the presence of CD8+/IFNG+ T cells. Conversely, 1 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.

Conclusions In 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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538 HARNESSING CROSS-DRESSING DENDRITIC CELLS TO STRENGTHEN ANTI-TUMOR IMMUNITY

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Background Cytotoxic ($CD8^+$) T-cells are required for tumor eradication and durable anti-tumor immunity.[1] The induction of tumor-reactive $CD8^+$ T-cells is predominately attributed to a subset of dendritic cells (DC) called Batf3-driven DC1, given their robust ability to cross-present antigens for T-cell priming and their role in effector T-cell recruitment.[2–4] Presence of the DC1 signature in tumors correlates with improved survival and response to immunotherapies.[5–7] Yet, most tumors with a DC1 infiltrate still progress, suggesting that while DC1 can initiate tumor-reactive $CD8^+$ T-cell responses, they are unable to sustain them. Therefore, there is a critical need to identify and engage additional stimulatory DC subsets to strengthen anti-tumor immunity and boost immunotherapy responses.

Methods To identify DC subsets that drive poly-functional $CD8^+$ T-cell responses, we compared the DC infiltrate of a spontaneously regressing tumor with a progressing tumor. Multicolor flow immunophenotyping and single-cell RNA-sequencing were used to profile the DC compartment of both tumors. IFNγ-ELISpot was performed on splenocytes to assess for systemic tumor-reactive T-cell responses. Sorted DC subsets from tumors were co-cultured with TCR-transgenic T-cells ex vivo to evaluate their stimulatory capacity. Cross-dressing (in vivo/ex vivo) was assayed by staining for transfer of tumor-derived $H\text{-}2^b$ MHC complexes to Balb/c DC, which express the $H\text{-}2^d$ haplotype. Protective systemic immunity was assayed via contralateral flank tumor outgrowth experiments.

Results Regressor tumors were infiltrated with more cross-presenting DC1 than progressor tumors. However, tumor-reactive $CD8^+$ T-cell responses and tumor control were preserved in $Batf3^{-/-}$ mice lacking DC1, indicating that anti-tumor immune responses could be induced independent of DC1. Through functional assays, we established that anti-tumor immunity against regressor tumors required $CD11c^+$ DC and cGAS/STING-independent type-I-interferon-sensing. Single-cell RNA-sequencing of the immune infiltrate of regressor tumors revealed a novel $CD11b^+$ DC subset expressing an interferon-stimulated gene signature (ISG^+ DC). Flow studies demonstrated that ISG^+ DC were more enriched in regressor tumors than progressor tumors. We showed that ISG^+ DC could activate $CD8^+$ T-cells by cross-dressing with tumor-derived peptide-MHC complexes, thereby bypassing the requirement for cross-presentation to initiate $CD8^+$ T-cell-driven immunity. ISG^+ DC highly expressed cytosolic dsRNA sensors (RIG-I/MDA5) and could be therapeutically harnessed by exogenous addition of a dsRNA analog to drive protective $CD8^+$ T-cell responses in DC1-deficient mice.

Conclusions The DC infiltrate in tumors can dictate the strength of anti-tumor immunity. Harnessing multiple stimulatory DC subsets, such as cross-presenting DC1 and cross-dressing ISG^+ DC, provides a therapeutic opportunity to enhance anti-tumor immunity and increase immunotherapy responses.