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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 adenocarcinoma colon and rectal from operative specimens. The blood level of CRP, IL-6, IFN γ , 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 signifiacnce difference were on the level of CRP, IL-6, IFN γ , 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 contrary, 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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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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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