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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	John Clyde Co Soriano	審査員主査： Chief Examiner	門之園 哲哉

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Myeloid cells are highly diverse immune cell populations that abundantly infiltrate tumors. Recent studies using single cell profiling technologies have highlighted their heterogeneity and found the existence of several unidentified cell populations. Among them, myeloid-derived suppressor cell (MDSC)-like adherent cells (MLACs) are a recently identified $CD11b^{+}F4/80^{-}$ myeloid cell subset that can infiltrate tumors early in development and promote tumor growth. But MLACs do not express immunosuppressive activity unlike MDSCs. Because of these functions, MLACs play an important role in establishing an immunosuppressive tumor microenvironment (TME). However, MLAC can only be isolated through adhesion-based separation, and share several surface markers with MDSCs such as Ly6C and Ly6G. Hence, the lack of MLAC-specific markers has hampered further characterization of this cell type. Furthermore, *in vivo* studies on the precise functions of MLACs have been limited.

In order to discover surface markers to isolate MLAC by fluorescence-activated cell sorting (FACS) and gain a deeper understanding of their functions, transcriptome analysis was performed through a combination of RNA-seq analysis and analyzing public single-cell (sc) RNA-seq data. Because of similarity in known surface markers between MLACs and MDSCs, the transcriptome profile of these two cell populations were compared and several upregulated membrane protein encoding genes for MLAC were obtained. Gene set enrichment analysis (GSEA) of these upregulated genes suggests that MLACs are involved in unique biological processes compared with MDSCs, such as inflammation through cytokine production and granulocyte migration. These results support the previous finding that MLACs can recruit MDSCs through specific secreted factors. The differentially expressed genes of MLACs were further applied to a public scRNA-seq dataset which allowed for the identification a cell population that corresponds to MLACs. These analyses revealed that MLACs are an independent cell population distinct from other intratumoral myeloid cells. This also identified *CD209a, Mgl2, CD74* and *Klrd1* as MLAC gene markers. Because genes were validated to be

consistently upregulated in MLACs compared to MDSCs, these genes were assigned as a MLAC gene signature; a set of genes that can identify cells as MLACs.

Since mRNA expression level does not always correlate to protein expression, membrane proteome analysis was also performed to aid in discovering high confidence surface markers for MLACs. Membrane proteome analysis was performed by isolating membrane proteins from MLACs and MDSCs and subjecting them to liquid chromatography tandem mass spectrometry (LC-MS/MS). Several membrane proteins were detected and their expression correlated to transcriptome analysis. Rank-rank hypergeometric overlap (RRHO) analysis was then performed as a statistical tool to combine and integrate proteome analysis with RNA-sequencing data, and obtain the top correlated membrane protein candidates from both analyses. The expression of candidate protein markers between MLAC and MDSC were then tested by flow cytometry analysis by staining with commercially available antibodies. H2-Ab1 (histocompatibility 2, class II antigen A, beta 1), a MHCII subunit and CD11c (integrin alpha X), an adhesion protein, were highly expressed in a significant population in MLACs while they were not expressed in MDSCs.

Instead of relying on adhesion-based separation, labeling of H2-Ab1 and CD11c through antibodies was used to directly isolate MLAC subsets from $CD11b^{+}F4/80^{-}$ cells, which also contained MDSC, by FACS. The $CD11b^{+}F4/80^{-}H2\text{-}Ab1^{+}$ and $CD11b^{+}F4/80^{-}CD11c^{+}$ MLAC subsets composed half of the MLAC population that was isolated based on their adhesion properties (Adh-MLACs).

To validate $CD11b^{+}F4/80^{-}H2\text{-}Ab1^{+}$ and $CD11b^{+}F4/80^{-}CD11c^{+}$ populations as equivalent to Adh-MLACs, the expression of MLAC gene signature and presence of functional activities of MLAC were assessed. By performing qPCR, these H2-Ab1 and CD11c expressing MLAC subsets expressed high levels of MLAC signature genes. The growth promoting activity of these MLAC subsets were confirmed by performing co-culture growth assay with Lewis lung carcinoma (LLC) and the absence of immunosuppressive activity was confirmed by co-culture immunosuppression assay with naïve $CD8^{+}$ T cells. These were comparable to the characteristics of Adh-MLAC. In contrast, the corresponding $CD11b^{+}F4/80^{-}H2\text{-}Ab1^{-}$ and $CD11b^{+}F4/80^{-}CD11c^{-}$ cell populations had low expression of MLAC signature genes, had weak cancer growth promoting activity and were immunosuppressive. This suggests that H2-Ab1 and CD11c can be used in the direct isolation of MLAC populations from tumors.

Further analysis of these $H2\text{-}Ab1^{+}$ and $CD11c^{+}$ subsets can advance our understanding of MLACs, and can lead to the development of therapeutic strategies to inhibit or delay the formation of an immunosuppressive TME. CD11c may be a mechanism for the strong adhesion properties of

MLACs. Membrane proteome analysis suggests that MLACs heterogeneous express surface proteins. This study facilitates an integrated understanding of heterogeneous intratumoral myeloid cells to provide deeper insights into the mechanisms of development of an immunosuppressive tumor microenvironment.

備考 : 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
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