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論文題目	PGE_2-EP2/EP4 signaling elicits immunosuppression by driving the mregDC-Treg axis in inflammatory tumor microenvironment. （ PGE_2-EP2 / EP4 シグナルは炎症性の腫瘍微小環境下で mregDC-Treg 軸経路を亢進させることにより免疫応答を抑制する ）		

（論文内容の要旨）

Inflammation is a fundamental defense mechanism that protect host from harmful stimuli by activating immune response. However, in the tumor microenvironment (TME), active inflammation does not promote immune activation but co-exist with immunosuppression. How TME manipulates such a paradox remains an enigma and holds a crucial clue in improving cancer immunotherapy. Immune dysfunction in TME is driven by the intricate interaction of tumor-infiltrating immune cells, stromal cells, and cancer cells via various mediators. Among these, prostaglandin E_2 (PGE_2) is a predominant inflammatory mediator generally released during active inflammation and has a potential to play a pivotal role in regulating immune response. This work addressed this issue by studying the role of PGE_2 in TME of mouse LLC1 tumor.

In LLC1 mouse syngeneic tumor model, inhibition of PGE_2 biosynthesis by COX1 and COX2 inhibitors or blocking of PGE_2 receptors, EP2 and EP4, led to suppressed tumor growth. Interestingly, direct in vitro incubation of LLC1 cells with EP2 antagonist (EP2i) and EP4 antagonist (EP4i) did not exhibit a direct tumor cell-killing potency. Furthermore, *Ptger2* (encoding EP2) and *Ptger4* (encoding EP4) were found to be highly expressed in immune cells compared to stromal and cancer cells, suggesting an immune modulation effect of PGE_2 via EP2 and EP4 receptors.

To dissect the role of PGE_2/EP2-EP4 signaling in immune modulation, LLC1 tumor-bearing mice were treated with vehicle, EP2i, EP4i, or EP2i+EP4i and tumor-infiltrating immune cells underwent single-cell transcriptomic profiling using 3' Chromium 10X scRNA-sequencing assay. Unsupervised clustering revealed the immune landscape in LLC1 TME, including distinct subtypes of dendritic cells, lymphocytes, and tumor-infiltrating myeloid cells (TIM). Among these diverse immune cell types, mregDC (mature DC enriched in immunoregulatory molecules), a recently defined DC subtype was also identified in LLC1 TME.

Differential gene expression analysis highlighted 3 different pro-tumoral mechanisms of PGE_2. Firstly, PGE_2-EP2/EP4 activates NFkB pathway by inducing transcription of NFkB components in all three subtypes of TIM. Consistently, NFkB target genes such as *Ptgs2*, *Il1b*, *Hif1a*, and *Vegfa* were upregulated by PGE_2 and suppressed by EP2i+EP4i. This result suggests that PGE_2 drives pro-inflammatory response and angiogenesis in via NFkB activation in TIM. Secondly, PGE_2 upregulated Treg-recruiting chemokines, *Ccl22*, and *Ccl17* in mregDC, which can be reversed by EP2i+EP4i treatment. Administration of Ccl22 and Ccl17 neutralizing antibodies to LLC1 tumor-bearing mice reduced tumor growth and number of tumor-infiltrating Treg compared to total immune cells. Therefore, this result suggests the novel and critical role of mregDC in regulation of Treg trafficking to TME and fostering immunosuppression. Lastly, PGE_2 promotes Treg function by upregulated Treg activation signature genes expression via EP2 and EP4. In vitro, PGE_2 upregulated expression and increased stability of *Foxp3* in *in vitro* induced Treg differentiation.

（論文審査の結果の要旨）

本論文は、腫瘍微小環境（TME）で産生されるプロスタグランジン E_2（PGE_2）を軸に、TME での活発な炎症に拘らず免疫が抑制される謎の解明を試みた。このため、著者らは、LLC1 マウス腫瘍の増殖が PGE_2 受容体 EP2 と EP4 の選択的拮抗薬で抑制され、これが、浸潤免疫細胞への作用によることを確かめた後に scRNAseq を用いてその機構を解明した。この結果、まず、PGE2-EP2/EP4 が、腫瘍浸潤性骨髄細胞に働き NFkB を活性化して、*Il1b*、*Ptgs2*、および *Cxcl2* などの炎症関連遺伝子や *Vegfa*、*Hif1a*、および Osm といった血管新生関連遺伝子を亢進させ炎症と血管新生を促進すること、一方、同じ PGE2 シグナルが、TME の mregDC に働き *Ccl17* と *Ccl22* の発現を亢進させ TME での Treg 集積を促進すること、さらに、浸潤 Treg に働き Foxp3 やその他の活性化関連遺伝子の発現を亢進させることを見出した。最後に、この 2 つの PGE2 シグナルモチーフがヒト腫瘍でも保存されており、その程度が予後と相関することを明らかにした。

以上の研究は、腫瘍微小環境の病態解明に貢献しがん薬理学に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　６年　１月　２９日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

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