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論文題目	Targeted epigenetic induction of mitochondrial biogenesis enhances antitumor immunity in mouse model （マウスモデルにおいてエピジェネティックなミトコンドリア生合成の増強が引き起こす抗がん免疫の促進）		

（論文内容の要旨）

Cancer immunotherapy by blockade of PD-1 (an immunoinhibitory receptor expressed on activated T cells) has gained exponential interest over the last decade because of long-term clinical benefits, and effectiveness over a wide-range of cancer types. However, almost half of the cancer patients show less or no response to PD-1 blockade-based monotherapy. This limitation substantiates the need of developing small-molecule based therapeutic drugs that aid to functions of effector T cells and synergize with PD-1 blockade. Recent reports suggest critical role of mitochondria in deciding T cell priming, activation and fate determination. In addition, effector T cells become less active with reduced PGC-1 expression (a known master regulator of mitochondrial metabolism and biogenesis) in the suppressive tumor microenvironment. This suggest that enhancing mitochondrial activation and biogenesis in CD8+ T cells could be an effective approach to enhance efficacy of PD-1 blockade therapy. Pyrrole–imidazole polyamides (PIPs) are small DNA ligands that can be pre-programmed to recognize target DNA sequence and guide epigenetic modulators to control the gene expression. In this study, an epigenetic modulator termed EnPGC-1 (BI-PIP) was designed to augment the expression of PGC-1α/β. EnPGC-1 functions as a bifunctional recruiter where the BI (bromodomain inhibitor) moiety of EnPGC-1 recognizes and recruit the bromodomain (BD) of p300 and further the histone acetyl transferase (HAT) domain of p300 perform locus-specific acetylation in the PIP-binding region of PGC-1α/β. In the *in vitro* cell based evaluation, EnPGC-1 shown to enhance transcript and protein level of PGC-1α and PGC-1β in murine primary CD8+ T cells. EnPGC-1 enhances mitochondrial activation parameters (e.g., mass, potential, mSox) as well as mitochondrial respiration (measured by oxygen consumption rate, OCR). ChIP-sequencing analysis of H3K27Ac revealed a notable enrichment in the promoter region of both PGC-1α and PGC-1β that overlaps with EnPGC-1 binding sites. Encouraged with *in vitro* findings, we further tested whether EnPGC-1 synergize with PD-1 blockade therapy. EnPGC-1 in combination with PD-1 blockade accelerates the tumor clearance as well as improves the overall survival in MC38 tumor-bearing hosts compared to PD-1 blockade alone or untreated group. Analysis of immune effector CD8+ T cells from draining lymph node and tumor mass post therapy shows better mitochondrial activation, mitochondrial respiration, fatty acid oxidation, and more T cell infiltration with enhanced effector functions (e.g., IFN gamma expression) to the tumor mass in the combination group. The increment in OCR/ECAR ratio supports a feature of long-live memory like population which is also evident by enhanced Bcl2 level, enhanced spare respiratory capacity and fatty acid oxidation in CD8+ T cells in combination group over PD-1 blockade alone. Moreover, genome-wide gene expression analysis in the *in vitro* treated CD8+ T cells suggest EnPGC-1 mediated PGC-1α/β upregulation drives differential gene expression that regulates T cell activation, proliferation, OXPHOS and FAO in CD8+ T cells compared to control groups. The data generated by independent lines of evidence, suggested the recruitment of p300 acetyltransferase as the potential mechanism of EnPGC-1-mediated gene activation. This study underscores the novel clinical application of first DNA-based designer molecule, EnPGC-1, for therapeutic purposes in the cancer treatment of less responsive cancer patients by combination therapy of EnPGC-1 with PD-1 blockade.

（論文審査の結果の要旨）

PD-1 免疫チェックポイント阻害に基づく癌免疫療法は、有力な治療法であるがおよそ半数の患者はこの治療に反応しない。ミトコンドリアは、免疫をになう T 細胞の活性化と機能において重要な役割を果たしている。PGC-1 は、ミトコンドリアの機能と生合成を調節しており、その発現上昇は T 細胞を活性化させ抗腫瘍効果を増強させる可能性がある。本論文では、PGC-1α/βの発現を増強する EnPGC-1 と呼ぶ DNA 結合分子を設計し、ミトコンドリア機能の増強を評価した。EnPGC-1 で処理したマウス CD8T 細胞は PGC-1α/β発現が増強され、ミトコンドリア機能と生合成を増強した。PGC-1α/βのプロモーター領域の H3K27 アセチル化が遺伝子活性化のメカニズムであることが示された。マウス CD8T 細胞のトランスクリプトーム解析の結果、PGC-1α/βの発現上昇は、T 細胞の活性化、増殖に関連する遺伝子群を活性化することが示された。さらに、EnPGC-1 と PD-1 阻害の組み合わせは、効果的な腫瘍退縮を引き起こし、PD-1 単独と比較して担癌マウスの生存を延長した。要約すると、今回開発した EnPGC-1 は、PD-1 阻害と相乗的に作用し CTL の殺腫瘍効果を高めることが示された。本研究は、PD-1 阻害による癌免疫療法と組み合わせることにより反応性の低い癌患者を治療するための新しい道を開く可能性を示した。したがって、本論文は博士（医学）の学位論文として価値のあるものと認める。なお、本学位授与申請者は、令和３年　11 月　８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。