論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	HUGH SHUNSUKE COLVIN
論文題名 Title	Oncometabolite D-2-Hydroxyglurate Directly Induces Epithelial-Mesenchymal Transition and is Associated with Distant Metastasis in Colorectal Cancer (オンコメタボライトD-2-Hydroxyglurateは上皮間葉転換を誘導し、大腸癌の遠隔転移に関わる)

論文内容の要旨

〔目 的(Purpose)〕

Colorectal cancer is the third most common cancer and the fourth most common cause of cancer related deaths worldwide, leading to approximately 700,000 deaths each year. The frequency with which the disease presents at an advanced stage, and therefore beyond the scope of curative surgical resection, underlies the high number of deaths and emphasises the need for novel treatment strategies, underpinned by a better understanding of the disease. Deranged metabolism is a hallmark of cancer, playing a significant role in driving the disease process. One such example is the induction of carcinogenesis by the oncometabolite D-2 hydroxyglutarate (D-2HG), which is produced by the mutated enzyme isocitrate dehydrogenase (IDH) occurring in subsets of leukaemias and brain tumours. While *IDH* mutations are rare in colorectal cancer, the recent revelation that 2HG levels were elevated in the azoxymethane mouse model of intestinal cancer, raised the possibility of 2HG levels being elevated in the human disease through an alternative mechanism. We therefore set out to examine the role of D-2HG and its enantiomer, L-2HG, in colorectal ccancer.

〔方法(Methods)〕

2HG was measured separately as D- and L-2HG from cell samples with gas chromatography-mass spectrometry, by derivatisation to (D and L)-O-acetyl-2-HG acid di-(D)-2-butyl esters. For the tissue samples, D-2HG was specifically measured using an enzymatic assay. D- or L-2HG were applied to cells at 250μM as octylesters, given they can permeate across the cell membrane, to be converted by intracellular esterases to 2HG. Quantitative PCR, immunoblotting, wound healing assay and invasion assay were used to assess epithelial-mesenchymal transition. Chromatin immunoprecipitation was performed to assess the histone modifications at the promoter regions of transcription factors associated with epithelial-mesenchymal transition (EMT). Sanger sequencing was performed to check for mutations of *IDH*. Gene knockdown experiments were performed using small interfering RNA or short hairpin RNA. All experimental methods involving human tissues were carried out in accordance with the guidelines and regulations approved by the Ethics Committee at the Graduate School of Medicine, Osaka University.

〔成績(Results)〕

Here, we find in colorectal cancer cells that even in the absence of *IDH* mutation, the levels of D-2HG and its enantiomer L-2HG were elevated through glutamine anaplerosis. D-2HG, but not L-2HG, increased the trimethylation of histone H3 lysine 4 at the promoter region of *ZEB1*, a master regulator of epithelial-mesenchymal transition (EMT), and increased the expression of the *ZEB1* gene to directly induce EMT in colorectal cancer cells. EMT promotes the ability of cancer cells to invade the local tissue and enter into the bloodstream, leading to distant organ metastasis. D-2HG levels were elevated in colorectal cancer specimens, particularly in those associated with distant metastasis, supporting the observations in vitro and implicating the contribution of D-2HG in metastasis, the major cause of death in this disease.

〔総 括(Conclusion)〕

We demonstrate D-2HG to be elevated in colorectal cancer and that it directly induces EMT in colorectal cancer cells, a phenotype associated with cellular invasion. The findings from human colorectal cancer specimens also support the significant role of D-2HG in promoting distant organ metastasis. Treatment strategies centred around reducing the levels of D-2HG or inhibiting its downstream effects in colorectal cancer could be effective.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

最近のがんの研究では、がん遺伝子やがん抑制遺伝子の異常と関連して、がん細胞の代謝が、がんの形成や進行に影響を及ぼすことが明らかになってきました。脳腫瘍や白血病の細胞では、エネルギー代謝に関与するイソクエン酸脱水酵素（IDH）の遺伝子が変異し、2-ヒドロキシ・グルタール酸（2HG）が大量に蓄積されます。２HGは、細胞をがんへと導く「造腫瘍性代謝物（オンコメタボライト）」として知られています。

本研究では、大腸がんの細胞にオンコメタボライトである２HGが蓄積していることを明らかにしました。この蓄積した２HG（D/L異性体）のうち、D型の２HG（D-2HG）は、エピゲノム（遺伝子の発現制御）の変化を誘導して、上皮－間葉転換（EMT）によって周りの細胞に浸潤し、血流に入り、離れた組織へのがん転移を引き起こすことを見出しました。研究グループは、さらに、臨床検体から得られたがん細胞を用いて、IDHの変異のない場合でも、D-2HGの濃度が通常の細胞と比べて高いことを示し、D-2HGのレベルが高いほど、がんのステージが高く、遠く離れた臓器等に転移（遠隔転移）している確率が高いことを示しました。

以上より、本研究の成果は転移を伴う大腸がんに対して新たな治療法を開発する上で、有用な手がかりになると考えられ、学位に値すると考える。