様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Elucidation of glutamine metabolism in tumor endothelial cells to develop novel anti-cancer drugs

研究課題名（英文）Elucidation of glutamine metabolism in tumor endothelial cells to develop novel anti-cancer drugs

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研究成果の概要（和文）：腫瘍血管内皮細胞（TEC）は腫瘍血管新生研究において重要な細胞である．TECは特異性を有しているため，副作用の少ない新規血管新生阻害剤の標的となりうる．本研究では，TECにおけるグルタミン代謝とミトコンドリアの役割を解析した．TECは正常血管内皮細胞よりミトコンドリアの数が少なく，酸化的リン酸化よりも解糖系からより多くのATPを生成していることがわかった．また，TECはグルタミン利用により，多くのグルタミン酸を産生した．スルファサラジン（SSZ）によるシスチン/グルタミン酸アンチポーターを阻害するとin vitro , in vivo 両方においてTEC増殖が抑制された．

研究成果の学術的意義や社会的意義
社会経済と人類の健康に癌が及ぼす悪い影響を取り除くためにも，癌の新しい治療戦略構築に関する研究の発展が望まれる．近年，癌細胞の代謝経路の標的化は創薬上重要な戦略と認識されている．本研究により，癌細胞のみならず腫瘍血管内皮細胞の代謝の特性も治療標的として有望であることが明らかになった．また，既存の血管新生阻害剤と異なり，より腫瘍血管に特異的な治療薬のための標的をこの研究にて提案することができた．このことにより，創薬研究分野に新たな知見を提供し，同時に新しい癌治療の展望をもたらした．

研究成果の概要（英文）：Tumor endothelial cells are the main focus of tumor angiogenesis studies. Compared to normal endothelial cells (NECs), TECs have a unique biology, which offers an explorable hub for new drug targets without the adverse side effects observed with common antiangiogenic drugs.
The study examined the roles of glutamine metabolism and mitochondria in TECs. TECs had fewer mitochondria than NECs and produced more ATP from glycolysis than oxidative phosphorylation. Spare respiratory capacity was lower in TECs than in NECs. Glutamine utilization by TECs produced larger quantities of glutamate than other glutaminolysis metabolites. Consistently inhibition of the cystine/glutamate antiporter Slc7a11 with sulfasalazine (SSZ) decreased TEC proliferation more significantly than NECs after 72h although glutathione levels were rapidly decreased in all cells upon exposure. In the mouse tumor models, SSZ treatment decreased both tumor angiogenesis and tumor growth.

研究分野：細胞生物学，癌生物学

キーワード：Tumor endothelial cell　Glutaminolysis　Glutamate　Glutathione　Tumor amgiogenesis　Oxidative stress　Sulfasalazine　Slc7a11/System -xc

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Tumor angiogenesis in essential to tumor growth and cancer therapy due to role of blood vessels transport and delivery of nutrients, drugs, and waste products. Tumor endothelial cells (TECs) are key to vessel formation and hence the target of most angiogenesis inhibitors. Current medications however have adverse effects on normal vessels. Therefore, new therapeutic strategies which focus on unexplored characteristics of TECs such as metabolism and mitochondria functions without harmful side effects are needed.

２．研究の目的

The aim of the study was to understand the process of glutamine metabolism and its importance to tumor endothelial cells, and to further identity unique areas in the glutaminolysis pathway present in the tumor endothelial cells that can be targeted to inhibit tumor growth and angiogenesis.

３．研究の方法

TECs and normal endothelial cells (NECs) were isolated from mouse tumor xenografts and tumor-free dermis, by mechanical disruption of cells and enzymatic digestion with collagenase. The dissociated cells were separated and purified sequentially by magnetic-activated cell sorting (MACS) and florescent-activated cells sorting (FACS) respectively. Using RT-qPCR the expression of endothelial cell markers in the isolated cells were confirmed. The metabolomics of TECs and NECs were measured by the CE-MS metabolomics with and without carbon13- labelled glutamine. Cellular mitochondria were observed by electron microscopy analysis. Cellular bioenergetics analysis with the Seahorse flux analyser was used to ascertain the ATP production in the cells. Cell proliferation was measured by the MTS assay.

４．研究成果

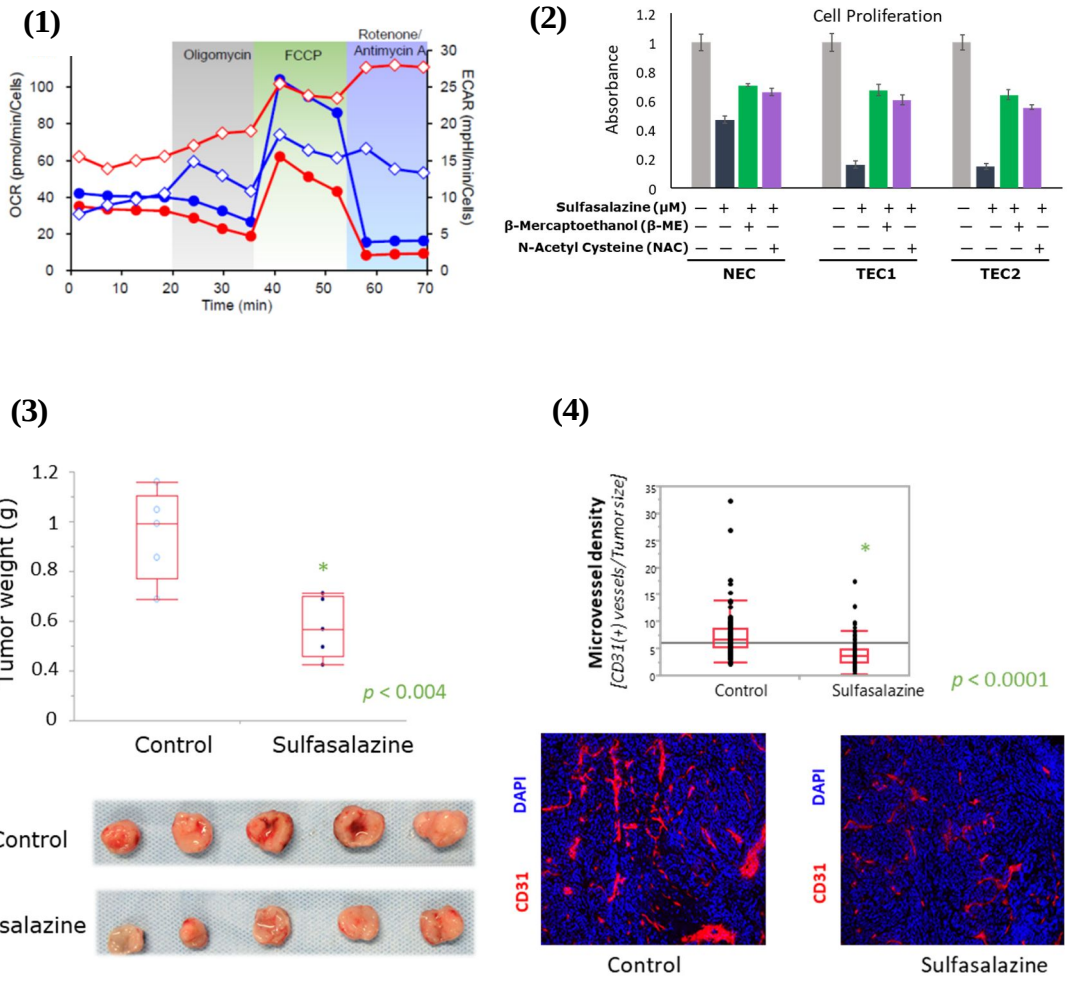
TECs and NECs showed different glutaminolysis fates. NECs converted glutamine primarily to GABA, whereas tumor endothelial cells produced more glutamate, asparagine, and aspartate from glutamine. TECs had fewer mitochondria content in the NECs were more than TECs. Cellular bioenergetics analysis revealed that the ‘extracellular acidification rate’ *(which is a measure of energy produced via glycolysis)* was higher in TECs than NECs (Fig.1). This observation suggested that glycolysis is the major ATP-producing process in the TECs. Conversely, in TECs, the ‘oxygen consumption rate’ *(an indicator of mitochondria respiration)* was lower than in NECs (Fig.1). Additionally, the ‘spare respiratory capacity’, which shows the bioenergetic reserve available to a cell under stressful conditions, was comparatively lower in TECs than NECs. The low spare respiratory capacity in TECs implies that TECs will be more susceptible to external insults than NECs.

Intracellular glutamate is exchanged for extracellular cystine across the plasma membrane via the sodium-independent glutamate/cystine antiporter Slc7a11*(also known as system xc-)*. This exchange facilitates the production of glutathione within cells and protection from oxidative stress. Both TECs and NECs expressed Slc7a11 messenger RNA and protein at comparable levels. Furthermore, taking into consideration the high levels of glutamate in TECs, it was wondered if Slc7a11 inhibition will cause significant harm to TECs, which already have a lower spare capacity. The drug sulfasalazine,

(Slc7a11 inhibitor and an anti-inflammatory medication for treating rheumatoid arthritis and ulcerative colitis) was selected to inhibit cystine exchange. Indeed, TEC proliferation was drastically reduced as compared to NECs (Fig.2). The antiproliferative effect of sulfasalazine was reversed by the addition of β-mercaptoethanol *(a reducing agent which allows cystine uptake via other transporters and also acts as an antioxidant)* (Fig.2). This implied that cystine uptake was essential for TEC survival. Cystine is the oxidized dimer of the cysteine amino acid, a rate-limiting factor to glutathione synthesis. N-acetylcysteine (NAC) provides cysteine to replenish the cysteine pool and sustain glutathione production. Treating TECs and NECs with NAC also abrogated the antiproliferative effects of sulfasalazine (Fig.2). In the presence of sulfasalazine, TEC mitochondrial respiration, extracellular acidification, and spare respiratory capacity were decreased, implying a general inhibition to metabolism and stress management in the cells.

The effect of sulfasalazine on *in vivo* angiogenesis was investigated using melanoma tumor xenografts in nude mice. Sulfasalazine treatment decreased tumor weights in the tumor-bearing mice as compared to the control mice (Fig.3). Angiogenesis was significantly diminished in the tumors treated with sulfasalazine (Fig.4). Tumor vessel maturity and collagen deposition in the extracellular matrix were however unaltered by sulfasalazine treatment.

The inhibitory effect of sulfasalazine on angiogenesis may provide a therapeutic window for combination therapy to improve current therapeutic strategies through reduction of treatment dosage of traditional medications so that the treatment can proceed with a fraction of the side effects usually observed in patients given these drugs. Further studies into combining sulfasalazine with conventional anticancer drugs are the next direction for this research.



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２．論文標題 Carbonic anhydrase 2 (CAII) supports tumor blood endothelial cell survival under lactic acidosis in the tumor microenvironment	５．発行年 2019年
３．雑誌名 Cell Communication and Signaling	６．最初と最後の頁 169
掲載論文のDOI（デジタルオブジェクト識別子） 10.1186/s12964-019-0478-4	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Nako Maishi, Dorcas A. Annan, Hiroshi Kikuchi, Yasuhiro Hida, and Kyoko Hida	４．巻 11(10)
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〔図書〕　計0件

〔産業財産権〕

〔その他〕
北海道大学　大学院歯学研究院　口腔病態学分野 血管生物分子病理学教室
https://www.den.hokudai.ac.jp/vascular-biol-pathol/

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関