HOKKAIDO UNIVERSITY

Title	Tumor endothelial cells survive in lactic acidosis via the activity of pH regulators [an abstract of dissertation and a summary of dissertation review]
Author(s)	ANNAN, DORCAS AKUBA-MUHYIA
Description	配架番号：2426
Degree Grantor	北海道大学
Degree Name	博士(医学)
Dissertation Number	甲第13291号
Issue Date	2018-09-25
Doc URL	https://hdl.handle.net/2115/71898
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/4.0/
Type	doctoral thesis
File Information	Dorcas_Akuba-Muhyia_Annan_abstract.pdf, 論文内容の要旨

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

学位論文内容の要旨
(Summary of Dissertation)

博士の専攻分野の名称 博士（医学）　氏 名 DORCAS AKUBA-MUHYIA ANNAN

学 位 論 文 題 名

Tumor endothelial cells survive in lactic acidosis via the activity of pH regulators

（乳酸アシドーシス環境におけるpH調節機構を介した腫瘍血管内皮細胞の生存）

Background and objectives

Tumor growth depends on the process of angiogenesis. The tumor endothelial cells (TECs) present in the tumor blood vessels have alterations which makes them different from normal endothelial cells (NECs). A number of these changes are as a result of influences from tumor-derived factors, tumor hypoxia, and reactive oxygen species. Additionally, products of tumor metabolism may also influence the function of TECs in the tumor microenvironment. Tumor cells undergo Warburg glycolysis to yield lactic acid as the end-product instead of pyruvate. The effects of lactic acid on immune cells have been reported, however there are no available reports regarding endothelial cells, except studies describing the individual effects of lactate or acidity. The accumulation of lactic acid creates a condition termed lactic acidosis, which has been described as an oncologic emergency due to its association with poor prognosis and high mortality rates in patients. In this study, it was hypothesized that TECs possess unique properties that promote their survival in a lactic acid-rich environment.

Cancer cells have devised various ways to avoid the harmful effects of lactic acid. They show upregulated expression of proton-coupled monocarboxylate transporters (MCTs), such as MCT1 and MCT4, to help regulate intracellular lactate and proton levels. Moreover, to maintain a stable intracellular acid-base balance they exhibit upregulated expression of pH regulators, including sodium/hydrogen exchanger 1 (NHE1), sodium/bicarbonate (Na+/HCO3-) co-transporters, proton-sensing G-protein-coupled receptors (GPCRs), vacuolar ATPases (V-ATPase) and carbonic anhydrases (CAs) including CAIX, CAXII and CAII. The unique function of pH regulators in TECs has not been elucidated. This study aimed to investigate the role of pH regulators in the survival of TECs under lactic acidosis.

Materials and Methods

In this study, TECs isolated from A375-SM tumor xenografts and NECs from the dermis of nude mice were used. The metabolomes of TECs and NECs were analyzed by the capillary electrophoresis-mass spectroscopy (CE– MS). The mRNA expressions of pH regulators and transporters were determined by real time-quantitative polymerase chain reaction (RT-qPCR). Small interfering RNAs (siRNAs) of selected genes were used to target mRNA and the effect on TEC proliferation in nutrient-replete medium (containing glucose and glutamine), lactic acidosis (20mM lactate, pH 6.9), and lactosis (20 mM lactate, pH 7.3) was determined. Cell proliferation was measured either by cell counting or the MTS assay. *In vivo* expression of CAII in mouse and human renal cell carcinoma (RCC) tissues was determined by immunohistochemically or by immunofluorescence staining. To identify the factors that may be responsible for CAII upregulation in TECs, immortalized HMVECs (iHMVECs) were treated with tumor-conditioned media (CM). For pharmacological

inhibition assays, monocarboxylate transport was targeted with α-cyano-4-hydroxycinnamate (CHC) and CAs with acetazolamide. A combination of acetazolamide and Ki8751 (VEGFR2 receptor inhibitor) was also used to target TEC proliferation *in vitro*. The effect of CA inhibition on tumor angiogenesis was investigated by injecting A375-SM tumor-bearing mice with 80mg/kg acetazolamide daily for 10 days.

Results

Unlike NECs, TEC culture medium was acidic, and TECs were able to withstand decreasing extracellular pH conditions. TECs showed a more glycolytic metabolome and produced more lactate than NECs. TECs proliferated in lactic acidosis, whereas this condition did not support NEC proliferation. The analysis of pH regulators showed that MCT1 and MCT4 were both expressed in TECs. In lactic acidosis, both MCT1 and MCT4 knockdown decreased the proliferation of the TECs. TECs also showed an upregulated expression of CAII, CAIII, and NHA2 as compared to NECs. Further analysis showed that TEC proliferation was significantly decreased by NHA2 knockdown and more significantly by CAII knockdown. Only CAII knockdown and not NHA2 knockdown led to a decrease in TEC proliferation in lactic acidosis and lactosis. It was observed that CAII was expressed in the endothelium of A375-SM and stage III RCC tumor tissues. In the normal kidney tissues as well as a stage I RCC specimen, endothelial CAII expression was not observed. Furthermore, it was demonstrated that protein factors in the A375-SM tumor CM including VEGF contribute to CAII upregulation in TECs. In vivo treatment with acetazolamide neither affected tumor growth or angiogenesis. However, a combination of acetazolamide and Ki8751 decreased in vitro TEC proliferation.

Discussion

The findings of this study showed that TECs survive in lactate rich environments, and their growth is not stunted by low extracellular pH. Consequently, TECs proliferated in lactic acidosis. TECs also express various classes of pH regulators. Among them MCT1, NHA2, and CAII are required for cell proliferation under conditions of nutrient availability. In lactic acidosis MCT1 and MCT4 knockdown decreased TEC proliferation, however, CAII knockdown caused a more significant decrease in TEC growth. CAII is an endothelium associated CA, and in this study, its importance to TEC function, particularly proliferation has been shown. These findings may suggest a significant contribution of TEC-specific CAII to the in vivo angiogenesis process in tumors. Furthermore, it was observed that CAII was expressed in the tumor endothelium of a stage III tumor. Similar observation has been made in other studies which associated the malignant progression of meningiomas with CAII expression. Although only one specimen was analyzed it can be proposed that CAII may contribute to tumor malignancy in some types of cancer including RCC.

Conclusion

The present study has shown that CAII is required for TEC proliferation under lactic acidosis as well as in conditions of nutrient availability. Additionally, CAII may work together with other pH regulators like MCT4 and NHE1 to ensure TEC survival in lactic acidosis. These findings point to a potential role of pH regulation in facilitating angiogenesis *in vivo*, making CAII and intracellular pH control prospective targets of tumor anti-angiogenic therapy.