論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	張 黎
論文題名 Title	Apelin as a marker for monitoring the tumor vessel normalization window during antiangiogenic therapy (アペリンは抗血管新生療法による腫瘍血管正常化マーカーになる)

論文内容の要旨

〔目 的(Purpose)〕

Antiangiogenic therapy normalize the tumor vasculature in a short period of time, made tumor vessels become less leaky, less dilated, and less tortuous with a more normal basement membrane and greater coverage by pericytes and therefore increased tumor perfusion, decreased hypoxia and improved penetration of drugs in tumors. Therefore, during normalization window, antiangiogenic therapy combined with chemotherapy or radiation therapy may lead to more effective therapies for patients with cancer. However, there are currently no reliable predictors or markers reflecting this vessel normalization window during antiangiogenic therapy. The aim of this work is to identify the suitable markers to monitor the normalization window during antiangiogenic therapy.

〔方法ならびに成績(Methods/Results)〕

Methods: Mice bearing colon adenocarcinoma cell line HT29 were treated with a single injection of bevacizumab. Tumor growth, vessel density (CD31), pericyte coverage (α-SMA), tumor hypoxia (pimonidazole) and small molecule delivery (Hoechst 33342) were determined by immunofluorescence at the 4 different time points (days1, 3, 5 and 8) after bevacizumab administration. Apelin, the expression of which is regulated by hypoxia through HIF-1a, and which has well-described roles in tumor progression, is an easily measured secreted protein. After determination of vessel normalization window, we investigate the changes of apelin expression during normalization window after bevacizumab treatment.

Results: Bevacizumab delayed tumor growth at day 3, 5, and day 8, suggesting that bevacizumab alone also have antitumor effect on HT29 xenografts. Bevacizumab treatment significantly reduced vessel density at day 3 and persisted throughout the study period of 8 days. After bevecizumab treatment, vessels became less dilated and less tortuous at days 3 and 5 than control tumors, but vessels dilated again at day 8 and pericytes showed a more uniform arrangement surrounding the vessels and became proximal to vessels at day 3 and 5 compared with the tumors from control or day 8. Vessel maturity index significantly increased at days 3 and 5 after bevacizumab treatment. Tumor hypoxia and Hoechst delivery was improved at days 3 and 5 after bevacizumab treatment. These findings suggested that vessel normalization window occurred between days 3 and 5 after bevacizumab treatment in HT29 xenografts. Apelin mRNA expression and plasma apelin levels decreased transiently at day 5 post-treatment, coinciding with vessel normalization window. The expression of HIF-1a protein also had a transient significant decrease at day 5 after bevecizumab treatment. These data suggested that bevacizumab may indirectly, through HIF-1a activation by hypoxia, regulate the apelin expression.

〔総 括(Conclusion)〕

In conclusion, apelin is a potential indicator of the vessel normalization window during antiangiogenic therapy.

[Format-H07]

論文審査の結果の要旨及び担当者

（申請者氏名）張　黎

論文審査担当者	（職）	氏　名
主　査	大阪大学教授	島倉仰子
副　査	大阪大学教授	原　英二
副　査	大阪大学教授	岡田雅人

論文審査の結果の要旨

現行の、血管新生抑制剤によるがん治療では、この治療薬だけでは治療効果は観察されず、抗がん剤との併用により抗腫瘍効果がもたらされる。このことが基礎医学的に研究され、現行の血管新生抑制剤の効果は、透過性の亢進した、未成熟な腫瘍血管を正常化し、抗がん剤の腫瘍内への送達を改善する治療であることが判明してきた。しかし、この腫瘍血管の正常化については、血管新生抑制剤の投与後いつ生じるのかが、容易には判断できず、血液診断により血管正常化のタイミングが判断されるようになることに期待がもたれてきた。本研究では、腫瘍血管の正常化により、腫瘍内の低酸素状態が改善することに注目し、７回膜貫通型のG蛋白共役型受容体であるAPJの結合因子で、低酸素で血管内皮細胞にて発現の亢進するアペリンの発現を解析し、アペリン発現の低下が腫瘍血管の正常化のタイミングと一致することを見いだした。本研究は今後の腫瘍血管制御による治療に役立つ研究であり、学位に値するものと考えられる。