

Detection of metabolic changes in SCC tumor by mTOR inhibition using hyperpolarized ^{13}C -pyruvate MRI

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[Introduction] The mammalian target of rapamycin (mTOR) is a protein kinase that is centrally involved in the control of cancer cell metabolism, growth, and proliferation, and therefore has become an attractive therapeutic target. Inhibition of mTOR by rapamycin causes diverse effects on tumor microenvironment, such as angiogenesis and oxygenation, as well as tumor energy metabolism. Such changes can serve as useful markers to assess tumor response to therapy. Recent development of ^{13}C -MRI with hyperpolarized ^{13}C -labeled compounds enabled us to monitor metabolic changes in tumors *in vivo*. $[1-^{13}\text{C}]$ pyruvate has been frequently used in cancer studies as an imaging tracer to monitor glycolytic processes (1,2), since it is involved in important bioenergetic processes that are altered in cancers. In this study, we investigated effects of rapamycin on pyruvate metabolism in squamous cell carcinoma (SCC) using ^{13}C -MRI with hyperpolarized $[1-^{13}\text{C}]$ pyruvate.

[Methods] SCC cells (5×10^5 cells) were implanted subcutaneously into a right hind leg of female C3H mice, and treatment with rapamycin (10 mg/kg b.w./day) and MRI measurements were started after 8 days from implantation of the SCC tumor. $[1-^{13}\text{C}]$ pyruvate containing 15 mM OX063 was polarized for approximately 1 hour using a hyperpolarizer (HyperSense, Oxford Instruments), and the hyperpolarized $[1-^{13}\text{C}]$ pyruvate (300 μL of 96 mM solution) was injected intravenously to the tumor-bearing mice. MRI measurements were carried out with a 4.7 T scanner and a 7 T scanner controlled with ParaVision 5.1 (Bruker Bio-Spin MRI GmbH).

[Results and Conclusion] $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate were detected in a tumor-bearing leg immediately after hyperpolarized $[1-^{13}\text{C}]$ pyruvate injection (Figure 1A), indicating exogenously injected pyruvate was quickly converted to lactate by lactate dehydrogenase (LDH) catalyzed reaction in the SCC tumor. The formation of $[1-^{13}\text{C}]$ lactate was decreased by rapamycin treatment. $[1-^{13}\text{C}]$ lactate to $[1-^{13}\text{C}]$ pyruvate ratio calculated from the area under the curves decreased by 2 days of rapamycin treatment, whereas it increased as tumor grew in non-treated control mice (Figure 1B). MRS imaging study also showed lactate formation was dropped by 2 days of rapamycin treatment (Figure 1C). Independent *in vitro* experiments using SCC tumor cells revealed that LDH activity was significantly smaller in rapamycin treated tumors compared with control tumors, indicating the decrease of LDH activity is one of the factors of the drop of lactate formation observed in the ^{13}C -MRI. In conclusion, inhibition of mTOR pathway by rapamycin treatment causes decrease of LDH activity in SCC tumor, and lactate formation from pyruvate monitored using hyperpolarized ^{13}C -MRI would become a useful marker for tumor response to cancer treatment.

[References] [1] Golman K. Et al., *Can. Res.* **66** (2006) 10855-10860. [2] Day SE. et al., *Nat. Med.* **13** (2007) 1382-1387.

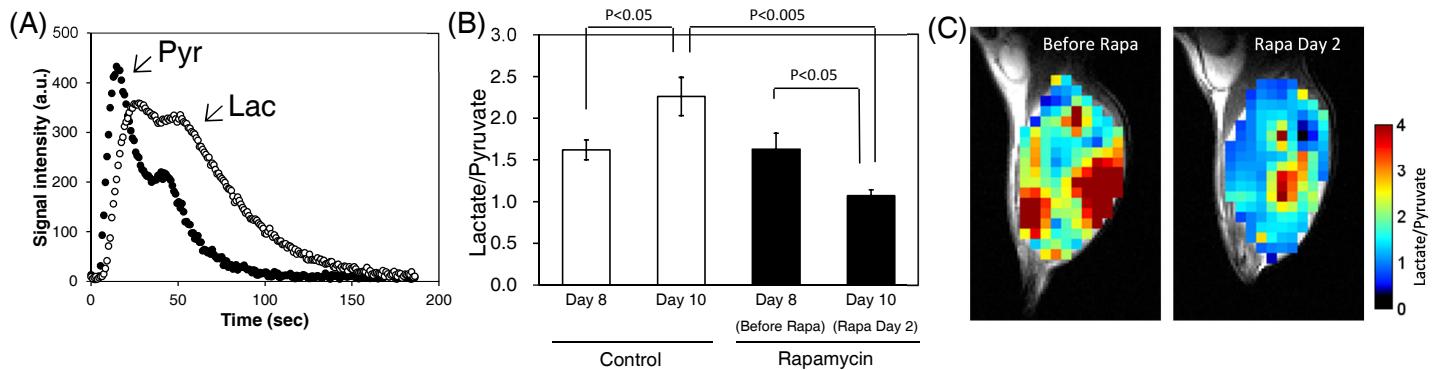


Figure 1 (A) Signal intensity changes of $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate in a SCC tumor. (B) $[1-^{13}\text{C}]$ pyruvate to $[1-^{13}\text{C}]$ lactate ratio calculated from the area under the curve of signal intensity curves. (C) $[1-^{13}\text{C}]$ lactate to $[1-^{13}\text{C}]$ pyruvate ratio maps measured before and after 2 days of rapamycin treatment. The imaging was started 30 sec after $[1-^{13}\text{C}]$ pyruvate injection, and it took 20 sec to obtain 1 image.