

Hyperpolarized ^{13}C -pyruvate MRI for evaluating metabolic changes in SCC tumor by X-irradiation

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[Introduction] X-irradiation to tumors causes diverse effects on tumor microenvironment, such as changes in perfusion and oxygen, as well as tumor metabolism. Such changes can serve as useful markers to assess tumor response to radiotherapy. 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 used in cancer imaging (1,2), since pyruvate is involved in important bioenergetic processes that are altered in cancers. In this study, we investigated effects of X-irradiation 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 MRI measurements and X-irradiation were started 8 days after the tumor cells implantation. $[1-^{13}\text{C}]$ 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 7 T scanner and a 4.7 T scanner controlled with ParaVision 5.1 (Bruker Bio-Spin MRI GmbH). A tumor-bearing leg was irradiated (10 Gy/day, total 30 Gy) using a XRAD-320 (Precision X-ray Inc.).

[Results and Conclusion] $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate were detected in the tumor-bearing leg immediately after hyperpolarized $[1-^{13}\text{C}]$ pyruvate injection (Figure 1A), indicating that exogenously injected pyruvate was quickly converted to lactate by lactate dehydrogenase (LDH) catalyzed reaction in the SCC tumor. $[1-^{13}\text{C}]$ lactate to $[1-^{13}\text{C}]$ pyruvate ratio calculated from the area under the curves increased as tumor grew in non-irradiated control group (Figure 1B). 10 Gy irradiation slightly suppressed the increase of lactate formation but there was no significance compared with the control group (Figure 1B, Day 9). However, lactate to pyruvate ratio significantly dropped 1 day after the third irradiation (total dose 30 Gy), and it was much smaller than the control group (Figure 1B, Day 11). MRS imaging studies also showed similar results; lactate formation increased after the first 10 Gy irradiation, and then dropped 1 day after 30 Gy irradiation (Figure 2). Independent *in vitro* measurements using SCC tumor homogenate revealed that LDH activity was significantly smaller in 30 Gy irradiated tumors compared with same day 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, X-irradiation suppressed increase of LDH activity in SCC tumor, and lactate formation from pyruvate would become a useful marker for tumor response to radiotherapy.

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

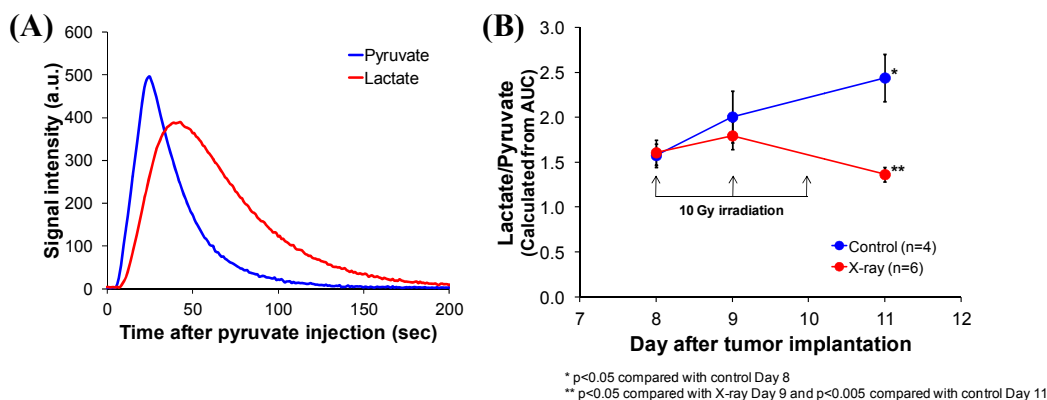


Figure 1 (A) Signal intensity changes of $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate in a SCC tumor after hyperpolarized $[1-^{13}\text{C}]$ pyruvate injection. (B) $[1-^{13}\text{C}]$ lactate to $[1-^{13}\text{C}]$ pyruvate ratio calculated from the area under the curves in (A). In X-irradiated group (red), Day 8 was measured before X-irradiation.

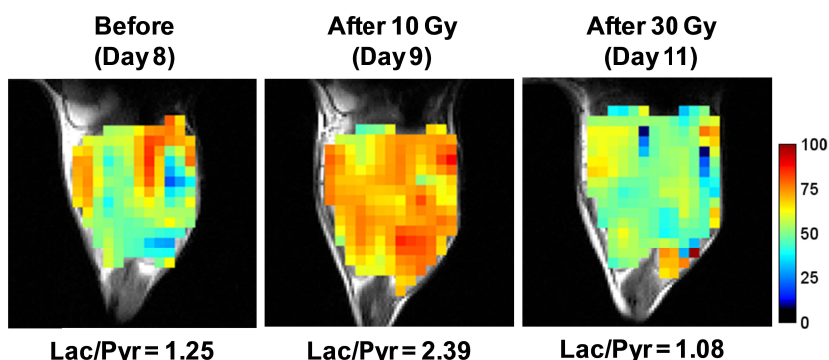


Figure 2 $[1-^{13}\text{C}]$ lactate to total ^{13}C ratio (%) maps measured before irradiation, 1 day after the first 10 Gy irradiation, and 1 day after the third irradiation (total dose 30 Gy). The measurement was started 30 sec after $[1-^{13}\text{C}]$ pyruvate injection, and it took 20 sec to obtain 1 image.