CHEMICAL SHIFT IMAGING APPLICATION OF TAGITININ C ANTIMETASTATIC ACTIVITY IN XENOGRAFT MODELS OF HEPATOCELLULAR CARCINOMA

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Purpose

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide. Metastases are the most common cause of death in cancer patients[1]. As a consequence the control of metastases formation has become one of the essential goals in cancer treatment. Much more research about anti-metastatic drugs had been done in this area within the last decades. Tagitinin C, a major sesquiterpenoid, was isolated from the leaves of *Tithonia diversifolia.* We have identified the anti-hepatoma ability of tagitinin C in previous data[2]. In this study the anti-metastasis ability of tagitinin C in xenograft models of hepatocellular carcinoma was investigated by chemical shift imaging (CSI), a non-invasive and more effective early detection method.

Methods

Cell lines and Tagitinin C: The human hepatoma cell line Hep-G2 (p53 wild type) maintained in Dulbecco-modified Eagle's medium (DMEM; Life Technologies, Grand Island, NY) were purchased from Food Industry Research and Development Institute (Hsin-Chu, Taiwan). Tagitinin C extracted from dried leaves of Taiwan *Tithonia Diversifolia* by 10~60% EtOAc/Hexaneon was used to test antimutagenic activity against Hep-G2 by mice xenografts. Five-week-old severe combined immunodeficiency (SCID) mice were purchased from Experimental Animal Center of Taiwan University (Taipei, Taiwan). Animal study: For tumorigenicity assays, mice were randomly subdivided into 2 groups (n = 5 for each group), and each mouse was injected subcutaneously at a single site with 1.5×10^7 Hep-G2 cells per mouse. The 0.075% dimeththyl sulfoxide (DMSO) or tagitinin C dissolved in 100 mg/μL DMSO (15 μg/mouse/day) was delivered intraperitoneally 2 days after the cell inoculation. Then mice were analyzed by Magnetic Resonance Spectroscopy (MRS) after 5–7 days following the drug administration. In vivo MRS Studies: 1.5 Tesla Magnetic Resonance Imager (Sonata, Siemens, Erlangen, Germany) was used to acquire the CSI data of the slice containing cell inoculation position of mice.

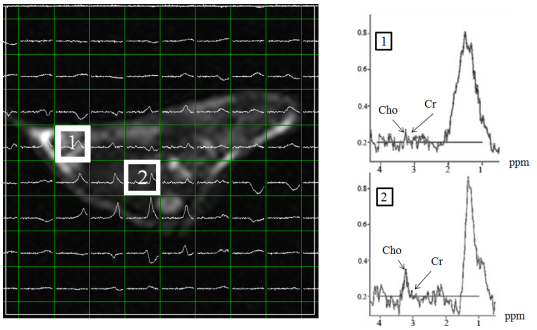

Fig1. Multi voxel spectra of Hep-G2 subcutaneous xenografts mouse after 25 days inoculation. Voxel 1 represented the inoculated point with Hep-G2 and voxel 2 showed maximum choline level.

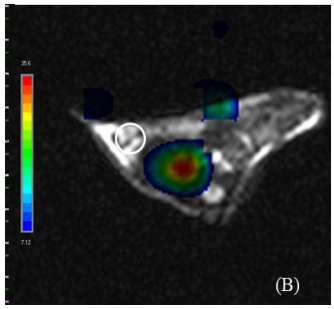

Fig2. The choline/creatine map of Fig1, indicated most of the cancer cells migrated from voxel 1 to voxel 2.

Results

After baseline and phase correction of MRS data (Fig. 1), the CSI choline/creatine maps (Fig. 2) were constructed and their integrations were calculated for comparison. Choline/creatine levels obtained from MRS in the inoculated point of xenograft mouse derived by hepatoma cells were very small (vehicle group), but shifted to other section (see Table 1), indicating the cancer cells metastasized to other parts (5 days ~ 25 days). In addition, the tumorigenisity of xenografts derived from the cells were probably anti-migrated by the delivery of tagitinin C (15 μg/mouse/day) relative to the control counterparts by CSI after 15~25 days' treatment. At the same time, serum AFP, GPT, and even tumor size (data not shown) showed worse distinction between tagitinin C-treated group and control group.

Table 1. Effects of tagitinin C (Tc) on AFP, GPT and choline /creatine (Cho/Cr) ratio in CSI of Hep-G2 xenograft.

day	Group	AFP	GPT	Cho/Cr (inoculated point)	Cho/Cr (maximum Cho/Cr in whole slice))
5	Vehicle	<0.61	136.0±46.6	0.93±0.93	55.81±16.02
	Tc	<0.61	29.3±5.9	11.52±4.80[a]	31.83±8.12[a]
15	Vehicle	890.0±191.7	28.4±2.5	0.00±0.00	72.40±9.70
	Tc	961.8±185.3	68.8±38.1	6.53±3.83[a]	29.32±6.30[a]
25	Vehicle	>12100	362.4±124.00[b,i]	0.15±0.15	34.49±5.52[b]
	Tc	>12100	74.3±27.00[c]	1.87±0.87[a]	13.19±2.52[c]

Discussion

Our CSI assay showed that tumor cells spread throughout all parts of the abdomen and metastasized to many organs of the mice. In our study, the finding of increased choline signal measured in a hepatoma tumor larger than 12 mm in diameter agreed with the finding of previous reports that choline reflects tumor cellular proliferation and grade of malignancy[3]. Thus, choline signal can be used as an indicator of tumor response after antitumor treatment when the present tumor model is applied. Our study had limitations. Since it was difficult to use breath-holding or respiratory gating techniques in mouse, respiratory movement during abdominal MR imaging might degrade image quality to some extent and cause variations in measurements. In addition, the size of mouse liver was too small to obtained sufficient SNR during a limited time period.

Conclusion

In conclusion, this liver tumor model was investigated successfully with MR imaging techniques including MRS with a clinical 1.5-T MR imager. Thus, the use of our model to study preclinical evaluation of new therapeutic drugs may provide an upgraded research platform.

References

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