中文題目：讓腎臟癌細胞中 SLC22A2 基因表現增加可提高 Metfromin 抗癌效果

英文題目：Re-enhancement of SLC22A2 gene in renal cell carcinoma could induce Metformin antineoplastic activity

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***Background**:*

Growing evidences suggest that metabolic disorder will further affect the prognosis of renal cell carcinoma (RCC), and Diabetes mellitus (DM) is one of the most common metabolic diseases. In one meta-analysis investigating the association of DM and RCC, DM significantly violate overall survival (HR 1.56, 95% CI, 1.35–1.81, P<0.001), cancer specific survival (HR 2.03, 95% CI, 1.37–3.01, P<0.001), and recurrence free survival (HR 1.73, 95% CI, 1.25–2.39, P=0.012)[1]. It is important to reverse the poor prognosis of RCC worsen by DM.

Metformin is a prevalent medication for type 2 DM. In recent time, epidemiological retrospective studies and in vitro studies both show that cancer patients may benefit from taking metformin as an adjuvant in chemotherapy, such as breast, colorectal and prostate cancer[2]. However, the evidence of metformin use and its survival benefit to RCC is sparse[3-6]. The possible reason may be due to the reduced expression of organic cation transporter 2 (OCT2) in RCC in comparison with normal kidney tissue[7]. Metformin is a hydrophilic organic cation and cannot cross cell membrane via passive diffusion. It is transported by different kinds of OCTs in human body[8]. OCT2 (gene SLC22A2) is exclusively expressed on the basolateral membrane of the proximal tubule in normal kidney tissue and accounts for metformin uptake into tubular epithelial cell[9]. RCC also originates from renal proximal tubule, however, almost no OCT2 expression can be detected in RCC compared with adjacent normal tissues. Epigenetic analysis in RCC shows suppression of OCT2 promoter, which is related to CpG islands hypermethylation and results in repressed gene transcription[7]. The suppression of OCT2 in RCC cells may inhibit the uptake of metformin and compromise its antineoplastic activity.

The purpose of our study is to know when RCC occurs in DM patients, whether we could utilize the potential anti-tumor activity of metformin as an adjuvant therapy. We investigated the pathophysiological role of OCT2 in the transportation of metformin in RCC, and the anti-RCC effect of metformin through OCT2 activation in vivo and in vitro.

Methods:

We first analyzed the expression of OCT2 in RCC from online database, and used the formalin-fixed paraffin-embedded section from clinical specimen of RCC patients in National Cheng Kung University Hospital to further confirm the reduced OCT2 expression by immunohistochemistry (IHC). Then we overexpressed the OCT2 in RCC cell line 786-O, and

examine whether the antineoplastic activity of metformin would be restored after OCT2 expression. In the next step, we confirmed our theory in animal model. Xenograft mice was established, and the mice were subcutaneously injected with 786-O-OCT2 as experimental group, and 786-O-GFP as control group. We examined the difference of metformin anti-tumor activity between these two groups.

Results:

●The expression of OCT2 was significantly decreased in patients with RCC

According to the analysis from Oncomine online data bank, the expression of OCT2 in the kidney from patients with RCC was significantly decreased (Figure 1). In order to confirm the results obtained from Oncomine, we collected the clinical specimen from patients with RCC, and then used IHC and Western blots to analyze the expression of OCT2. We found that the expression of OCT2 was detected dominantly in the proximal tubules of kidney. In addition, the levels of OCT2 were significantly decreased in RCC as compared with non-tumor part (Figure 2). Moreover, OCT2 expression decreased in the early stage of RCC, and showed no differences between different stages of RCC.

●Overexpression of OCT2 in 786-O RCC cells augmented the cytotoxicity effects of metformin

In order to evaluate the role of OCT2 in metformin-mediated anti-cancer activity, we used lentiviral vectors to overexpress OCT2 in 786-O RCC cells (Figure 3). As shown in (Figure 4), metformin exerted significant anti-cancer activity in OCT2-overexpressed 786-O RCC cells, as compared with vector-alone group, indicating the cytotoxicity of metformin was mainly through OCT2.

●Metformin exerted anti-cancer activity in OCT2-overexpressed 786-O RCC cells in vivo

In order to evaluate whether metformin showed anti-RCC activity in vivo, we established a RCC xenograft animal model. As shown in (Figure 5), 786-O RCC xenograft successfully implanted in mice, and metformin showed significantly anti-RCC activity in OCT2-overexpressed group as compared with GFP-control group.

Conclusion:

OCT2 is the main transporter for metformin uptake in renal tubular epithelial cell, and the expression of OCT2 significantly decreased in RCC. Decrement in OCT2 expression in RCC might diminish the anti-cancer activity of metformin.

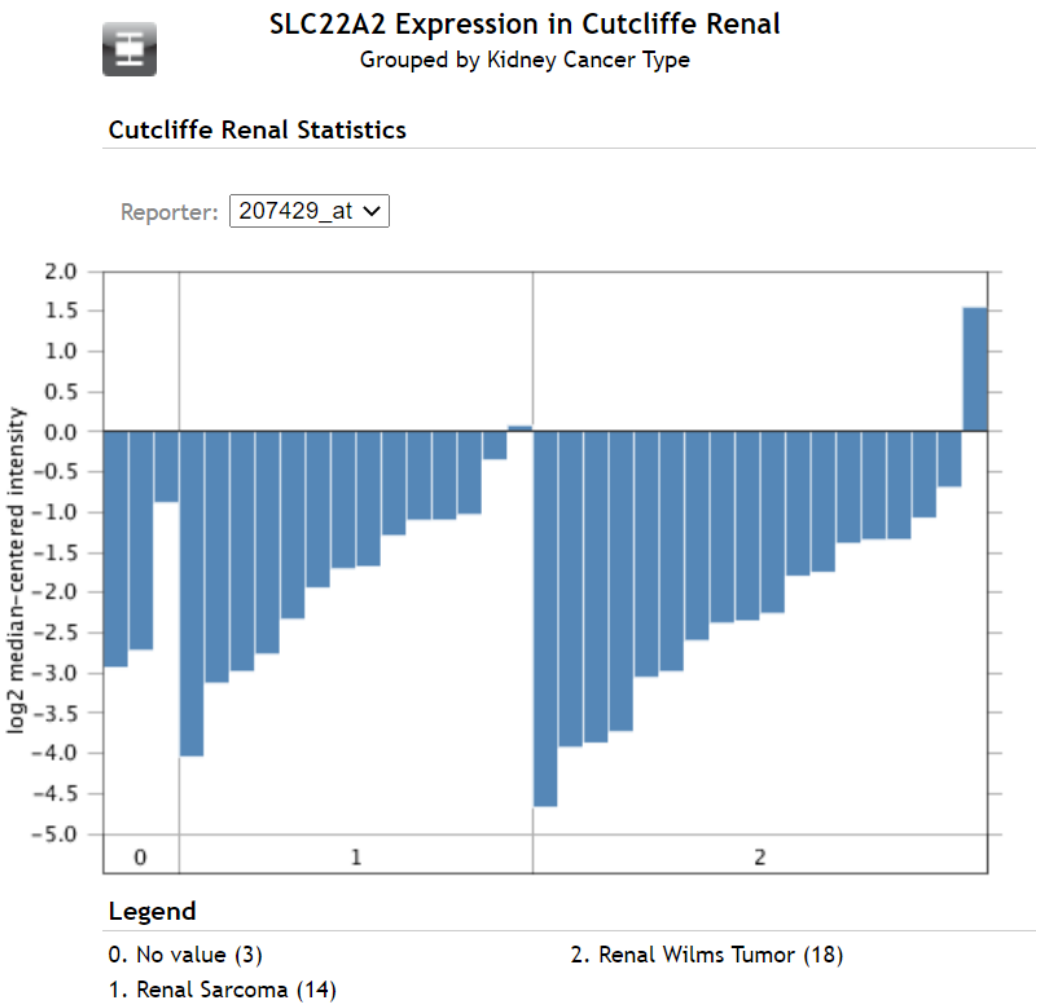

Figure 1. The analysis from Oncomine online data bank, the expression of SLC22A2 gene was significantly decreased in patients.

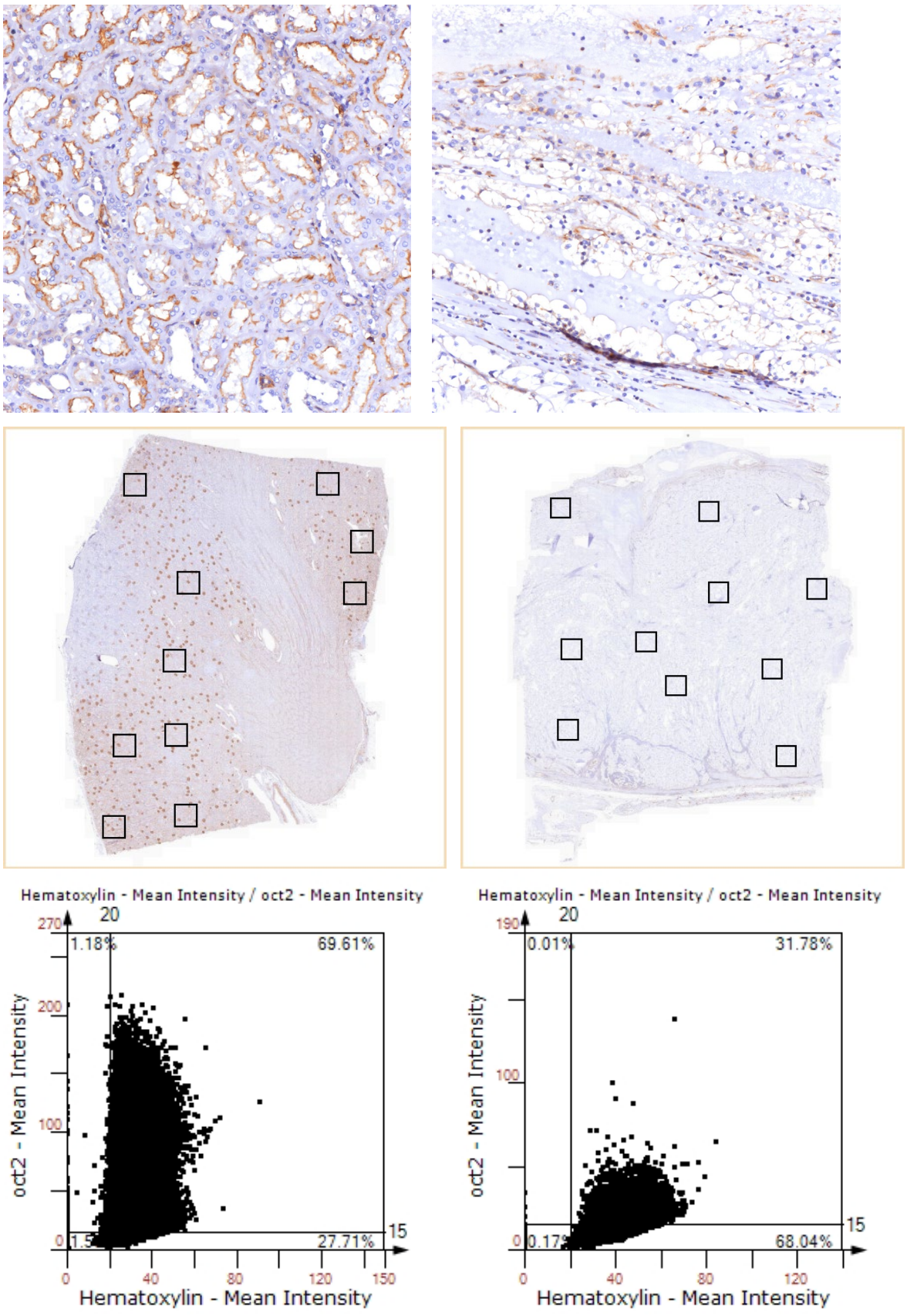

Figure 2. In the proximal tubules of kidney, we detected less OCT2 in tumor-part than that in normal-part.

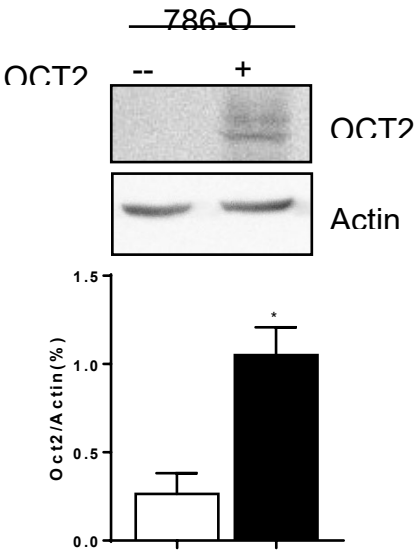

Figure 3. 786-O RCC cells were transfected with OCT2 overexpression plasmids.

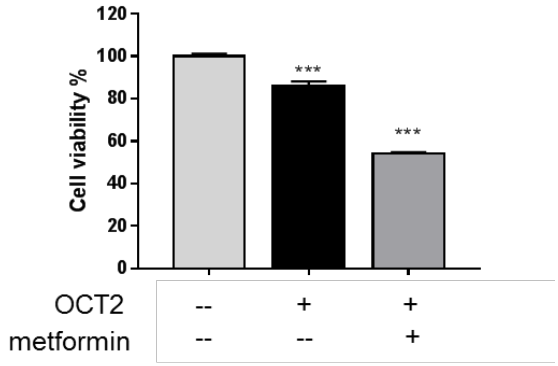

Figure 4. The cell viability of OCT2-overexpressed 786-O RCC cells treated with metformin was significantly decreased compared with cells without metformin treatment.

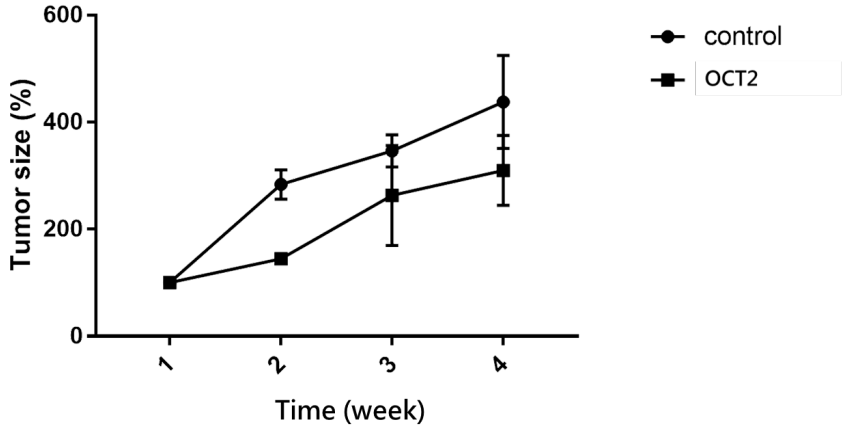

Figure 5. Under metformin treatment, the tumor size in OCT2-overexpressed 786-O xenograft is smaller than GFP-control.

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