MP01-09

Luteolin suppresses not only squamous differentiation of bladder cancer but cancer growth via regulation of mammalian target of rapamycin pathway

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Abstract

Introduction and Objective
Luteolin is a natural flavonoid with strong antioxidative properties. The anti-cancer effects of luteolin against several cancer have been reported, however, little is known about bladder cancer. Here, we determined to explore the anticancer effects of luteolin against bladder cancer.

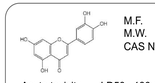
Methods
Human urothelial carcinoma cell line T24 and 5637 were used. WST8 assay and western blot analysis were used for evaluating cell viability and protein signaling. Thioredoxin activity and ROS production were evaluated using thioredoxin and DCFH-DA assays. Furthermore, we examined the impact of a 20-week luteolin rich diet on the growth of N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)–induced rat bladder cancer models (luteolin concentrations were control, 20ppm and 100ppm; n=20, respectively).

Results
Luteolin induced a dose-dependent reduction in the number of viable cells; it also increased thioredoxin activity and decreased intracellular ROS production. Luteolin downregulated phospho-p70S6K and phospho-S6K, which were substrates of mTOR, and they were canceled by thioredoxin inhibitor PX-12, indicating luteolin inhibited mTOR pathway through the regulation of thioredoxin and ROS. In *in vivo* study, BBN–induced rat bladder cancer was inhibited by the oral administration of luteolin and also showed a decreased Ki67-labeling index and p-S6 expression. Further, both plasma and urine luteolin-3'-*O* -glucuronide concentrations were strongly associated with the inhibition of cell proliferation (r=0.31, -0.41, respectively) and mTOR signaling (r=-0.60, -0.62, respectively). Moreover, a significant decrease in the squamous differentiation of bladder cancer is attributed to plasma luteolin-3'-glucuronide concentrations (p<0.01).

Conclusions
Luteolin may represent another natural product–derived therapeutic agent that acts against bladder cancer by up-regulating thioredoxin activity and inhibiting mTOR signaling.

Introduction and Objectives

- Smoking and aging are the major risk factor of bladder cancer and they are known to increase oxidative stress in normal cells and induce carcinogenesis.
- It has been reported that excessive ROS induces DNA damage, on the other hand, ROS has an important role as a cell proliferation signal. Schieber M. et al. Curr Biol. 2014
- Anti-cancer effects against several cancer have been reported, but the effect of luteolin on bladder cancer has received little attention in the literature. Wang L et al. Carcinogenesis. 2014 Naiki-Ito A et al. Carcinogenesis. 2015
- We explored the therapeutic effect of luteolin on bladder cancer.

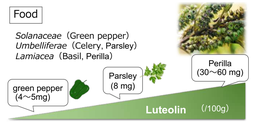


M.F. $C_{15}H_{10}O_6$
M.W. 286.24
CAS No. 491-70-3

Acute toxicity	LD50 : 180 mg/kg, i.p.
Mutagenicity	None (Mutat Res, 1990)
Carcinogenicity	None

Effect
- Anti-allergy, anti-inflammation and antioxidative effects
- Anti-cancer effect
(lung, colon, liver, ovary, breast, prostate, etc)

Food
Solanaceae (Green pepper)
Umbelliferae (Celery, Parsley)
Lamiacea (Basil, Perilla)



Methods

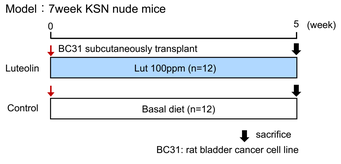
1. In vitro

We explored the mechanism of anti-cancer effect of luteolin on bladder cancer
<Cell lines> Human bladder cancer : T24, 5637
- Anti-proliferative effect
- The regulation of mTOR activity
- The regulation of intracellular ROS production and thioredoxin activity

<Experiments>
WST8 assay, Western blot, DCFH-DA assay ,
Thioredoxin assay, TCGA genome database

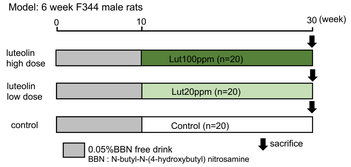
2. In vivo

Subcutaneous BC31 xenograft model



- Evaluate tumor volume and expression levels of Ki67and TUNEL, pS6.

BBN induced rat bladder cancer carcinogenesis model

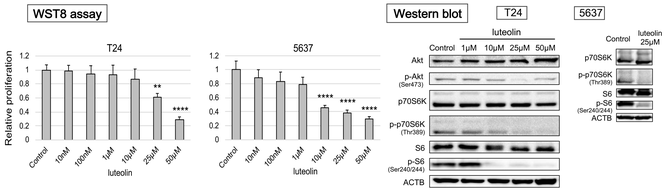


- Evaluate tumor dimension† and expression levels of Ki67and TUNEL, pS6, CK5/6.
- Measure the plasma and urine concentration of luteolin-3'-glucuronide by HPLC MS/MS. (control n=10, Lut 100ppm n=18)

Results

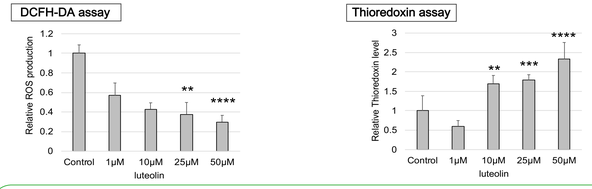
1. In vitro

Anti-proliferative effect

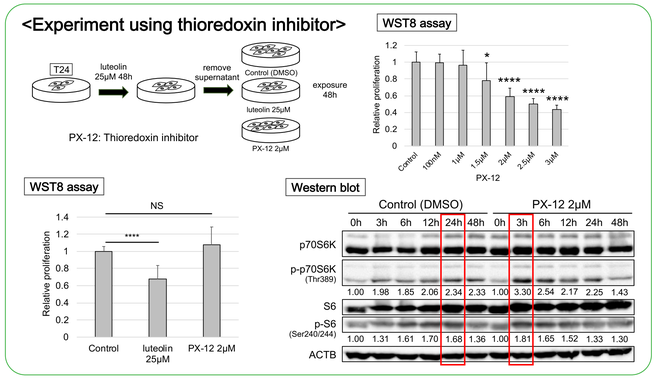


The regulation of mTOR activity

The regulation of intracellular ROS production and thioredoxin activity



<Experiment using thioredoxin inhibitor>

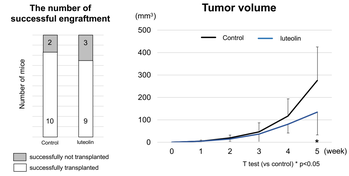


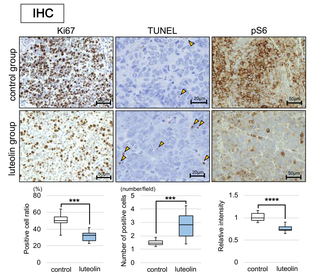
- Luteolin had anti-proliferative effect in concentration dependent manner.
- Luteolin increases thioredoxin activity and decreases intracellular ROS production.
- Experiment using thioredoxin inhibitor suggests that thioredoxin regulates mTOR activity.

2. In vivo

Subcutaneous BC31 xenograft model

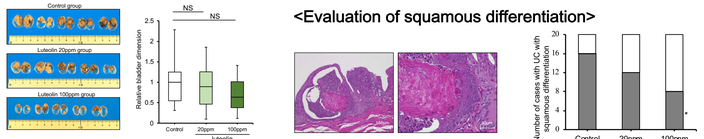
There were no differences in dietary intake and body, kidneys and liver weights. Histological analyses revealed no toxic effect was induced by luteolin. No metastasis were found.

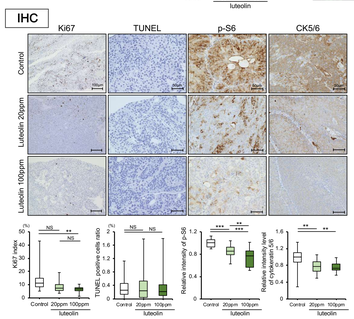




BBN induced rat bladder cancer carcinogenesis model

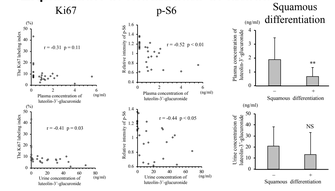
There were no differences in BBN intake and dietary intake, body, kidneys and liver weights.





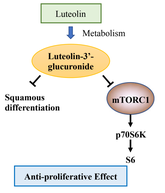
- mTOR inhibitory effect by luteolin intake were seen in both models.
- Luteolin also had an effect to prevent from inducing squamous differentiation.

<Exploration of metabolite of luteolin>



Conclusions

Luteolin, and in particular its metabolized product, luteolin-3'-glucrunide, may represent another natural product–derived therapeutic agent that acts against bladder cancer by inhibiting mTOR signaling.



COI Disclosure Information
Leader Presenter/Responsible Researcher
Keitaro Iida
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