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

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Abstract

**Introduction and Objectives**

Luteolin is a natural flavonoid with strong antioxidative properties. The anti-cancer effects of luteolin against several cancer have been reported, however, it is not known about bladder cancer. Here, we determined to explore the anti-cancer effects of luteolin against bladder cancer.

**Methods**

Human urethral carcinoma cell line T24 and 5637 were used. WST-8 and 4,5-diamino-2-methyl-3-(phenyltetrazolium bromide) (MTT) assays, and western blot analysis were used for evaluating cell viability and protein expression. Three-hour activity and ROS production were evaluated using thiosemicarbazide and DCFH-Da assays. Furthermore, we examined the impact of luteolin on metabolite (N-acetyl-p-butyryl-l-cysteine) and thioredoxin (BMR)-induced bladder cancer models (luteolin concentrations were: 20µM and 100µM, 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-pS6K and phospho-4EBP1, which were substrates of mTOR, and they were controlled by thioredoxin inhibitor PX-12, indicating luteolin inhibited mTOR pathway through the regulation of thioredoxin and ROS. In vivo study, BMM-induced rat bladder cancer was inhibited by the oral administration of luteolin and also showed a decreased Ki67-labeling index and p-STAT3 expression. Further, both plasma and urine luteolin in 10-20 ppm concentrations were strongly associated with the inhibition of cell proliferation (r = 0.31, 0.41, respectively) and mTOR signaling (r = 0.80, 0.62, respectively). Moreover, a significant decrease in the squamous differentiation of bladder cancer is attributed to plasma luteolin (10-20 ppm 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.

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**Results**

1. In vitro

**Anti-proliferative effect**

**The regulation of mTOR activity**

**The regulation of intracellular ROS production and thioredoxin activity**

**Experimental using thioredoxin inhibitor**

2. In vivo

**Subcutaneous BGC3 xenograft model**

**BMM induced rat bladder cancer carcinogenesis model**

**Evaluation of squamous differentiation**

**Exploration of metabolite of luteolin**

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**Conclusions**

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