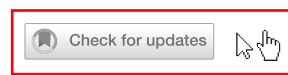


## ARTICLE OPEN



## Translational Therapeutics

# Nuplazid suppresses esophageal squamous cell carcinoma growth in vitro and in vivo by targeting PAK4

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**BACKGROUND:** Due to the high recurrence and low 5-year survival rates of esophageal squamous cell carcinoma (ESCC) after treatment, the discovery of novel drugs for recurrence chemoprevention is of particular importance.

**METHODS:** We screened the FDA-approved drug library and found that Nuplazid, an atypical antipsychotic that acts as an effective 5-HT 2 A receptor inverse agonist, could potentially exert anticancer effects in vitro and in vivo on ESCC.

**RESULTS:** Pull-down results indicated that Nuplazid binds with p21-activated kinase 4 (PAK4), and a kinase assay showed that Nuplazid strongly suppressed PAK4 kinase activity. Moreover, Nuplazid exhibited inhibitory effects on ESCC in vivo.

**CONCLUSIONS:** Our findings indicate that Nuplazid can suppress ESCC progression through targeting PAK4.

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## BACKGROUND

Esophageal cancer is the seventh most commonly diagnosed cancer and the sixth most common cause of cancer-related mortality worldwide [1]. Esophageal squamous cell carcinoma (ESCC) accounts for ~90% of esophageal cancers [2], and is typically treated with surgery, chemotherapy, radiotherapy and combination therapy [3, 4]. However, even with treatment, the 5-year overall survival rate has been reported to be ~47%, with 49% of patients developing locoregional progression or distant progression [5, 6]. Therefore, it is important to identify novel drugs with low toxicity and high efficacy to prevent the recurrence of ESCC and increase the survival rate of patients. The repositioning of drugs can overcome the challenges of high wastage, high cost, and time-consuming drug development [7, 8]. Screening drugs approved by the FDA and repositioning their anti-tumour effects have the potential to overcome several challenges associated with drug development and to guarantee rapid clinical trials.

p21-Activated kinase 4 (PAK4) is involved in numerous signaling pathways and plays a pivotal role in cytoskeleton regulation, cell migration, growth, proliferation and survival [9]. The overexpression of PAK4 is reported to be closely related to the occurrence and development of various cancers, including pancreatic [10], breast [11, 12], ovarian [13], and gastric cancers [14]. Currently, several compounds have been identified as PAK4 inhibitors, which typically target the ATP-binding pocket of PAK4 kinase domain [15]. However, the transition from compound inhibitors to clinical medications needs to overcome time-consuming and exorbitant cost factors.

Nuplazid, an atypical antipsychotic that functions as an effective 5-HT 2 A receptor inverse agonist, is mainly used to treat Parkinson's disease psychosis [16]. In this study, we screened drugs approved by the FDA and found Nuplazid could inhibit the growth of ESCC in vitro. Then our study found that Nuplazid treatment inhibits the growth of ESCC by binding to PAK4 and regulating its downstream signaling pathway interaction. The anticancer effects of Nuplazid on ESCC in vitro and in vivo suggested Nuplazid might be a candidate for ESCC chemoprevention.

## METHODS

### Cell culture

The Shantou human embryonic esophageal (SHEE) cell line was obtained from Dr. Enmin Li (Medical College of Shantou University) [17]. Human esophageal cancer cell lines KYSE150, KYSE410 and KYSE450 cells were purchased from the Type Culture Collection of the Chinese Academy of Science. ESCC cells were cultured in RPMI-1640 medium (Biological Industries, China) supplemented with 10% inactivated FBS (Biological Industries, China) and 1% penicillin/streptomycin. The cells were cytogenetically tested by STR-Promega and were authenticated (August, 2014 and July, 2017) [18, 19]. HEK293T cells (ATCC) were cultured in DMEM medium (Biological Industries, China). All cells were maintained at 37 °C in a humidified 5% CO<sub>2</sub> incubator.

### Reagents and antibodies

Nuplazid was purchased from J&K Chemical (Beijing, China). jetPRIME® transfection reagent was purchased from Polyplus Transferion® SA. Protein

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