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Novel compounds TAD-1822-7-F2 and F5 inhibited HeLa cells growth through the JAK/Stat signaling pathway

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ABSTRACT

Cervical carcinoma remains the second most common malignancy with a high mortality rate among women worldwide. TAD-1822-7-F2 (F2) and TAD-1822-7-F5 (F5) are novel compounds synthesized on the chemical structure of taspine derivatives, and show an effective suppression for HeLa cells. Our study aims to confirm the potential targets of F2 and F5, and investigate the underlying mechanism of the inhibitory effect on HeLa cells. In this study, Real Time Cell Analysis and crystal violet staining assay were conducted to investigate the effect of F2 and F5 on HeLa cells proliferation. And the analytical methods of surface plasmon resonance and quartz crystal microbalance were established and employed to study the interaction between F2 and F5 and potential target protein JAK2, suggesting that both compounds have strong interaction with the JAK2 protein. Western blot analysis, immunofluorescence staining study and PCR was conducted to investigate the molecules of JAK/Stat signaling pathway. Interestingly, F2 and F5 showed diverse regulation for signaling molecules because of their different chemical structure. F2 increased the expression of JAK2 and downregulated the level of P-JAK1 and P-JAK2, and decreased P-Stat3 (Ser727). While F5 could increase the expression of JAK2 and naturally decrease the phosphorylation of JAK1 and Tyk2, and decreased the expression of P-Stat6. Moreover, F2 and F5 showed the same downregulation on the P-Stat3 (Tyr705). Therefore, F2 and F5 could target the JAK2 protein and prevent the phosphorylation of JAKs to suppress the phosphorylation of the downstream effector Stats, which suggested that F2 and F5 have great potential to be the inhibitors of the JAK/Stat signaling pathway.

1. Introduction

Cervical carcinoma is the second most common malignancy among women in the world and the age of onset is declining recent years [1]. The therapeutic methods for cervical cancer include surgery, medication, radiotherapy, heat treatment and gene therapy. Surgery combined with concurrent chemo-radiotherapy can cure 80%–95% early cervical cancer patients, but the treatment effect is not satisfactory for advanced and metastatic cervical cancers [2]. Therefore, new and efficient chemotherapeutics are in great need for the treatment of cervical cancer.

The JAK/Stat signaling pathway is critical for signal transmission of a wide array of cytokines and growth factors and mediates a lot of biological reactions including differentiation, migration, apoptosis and immune regulation, etc. [3]. Over activation of the JAK/Stat signaling pathway will promote the occurrence and development of solid tumors, lymphoma, leukemia and inflammatory disorders [4]. When the ligand binds to the receptors in the JAK/Stat signaling pathway, the receptors dimerized and be activated by the alternated phosphorylation of the tyrosine residue from link-coupled JAKs. The phosphorylation of the receptor recruits the STAT proteins which are phosphorylated by the JAKs [5], and then Stats combined with the receptors through the SH2 structural domain and transferred to the nucleus in a homo/hetero dimer manner. Afterwards, Stats bound to the promoter of the targeted gene and regulate the transcription and expression of the downstream molecules [6]. In mammals, 4 JAKs and 7 Stats were comprised and among them, Stat3 is closely related to tumor [7]. The unphosphorylated Stat3 as well as P-Stat3 can bind directly to DNA in order to play a role in gene regulation and can be a novel drug target in oncology [8], but most of Stat3 drug targeted to the phosphorylated Stat3 protein [9]. The activated Stat3 could promote tumor angiogenesis and accelerate the epithelial mesenchymal transition process through the JAK/Stat signaling pathway [10]. HPV16/18 positive cervical cancer patients are in high expression of Stat3, such as HeLa is positive of HPV18 [11]. Furthermore, endometrial and cervical cancer patients in

Abbreviations: RPMI, Roswell Park Memorial Institute; FBS, fetal bovine serum; IC50, 50%-growth inhibitory concentrations; RTCA, Real Time Cell Analysis; SPR, surface plasmon resonance; QCM, quartz crystal microbalance; K_D, equilibrium dissociation constant

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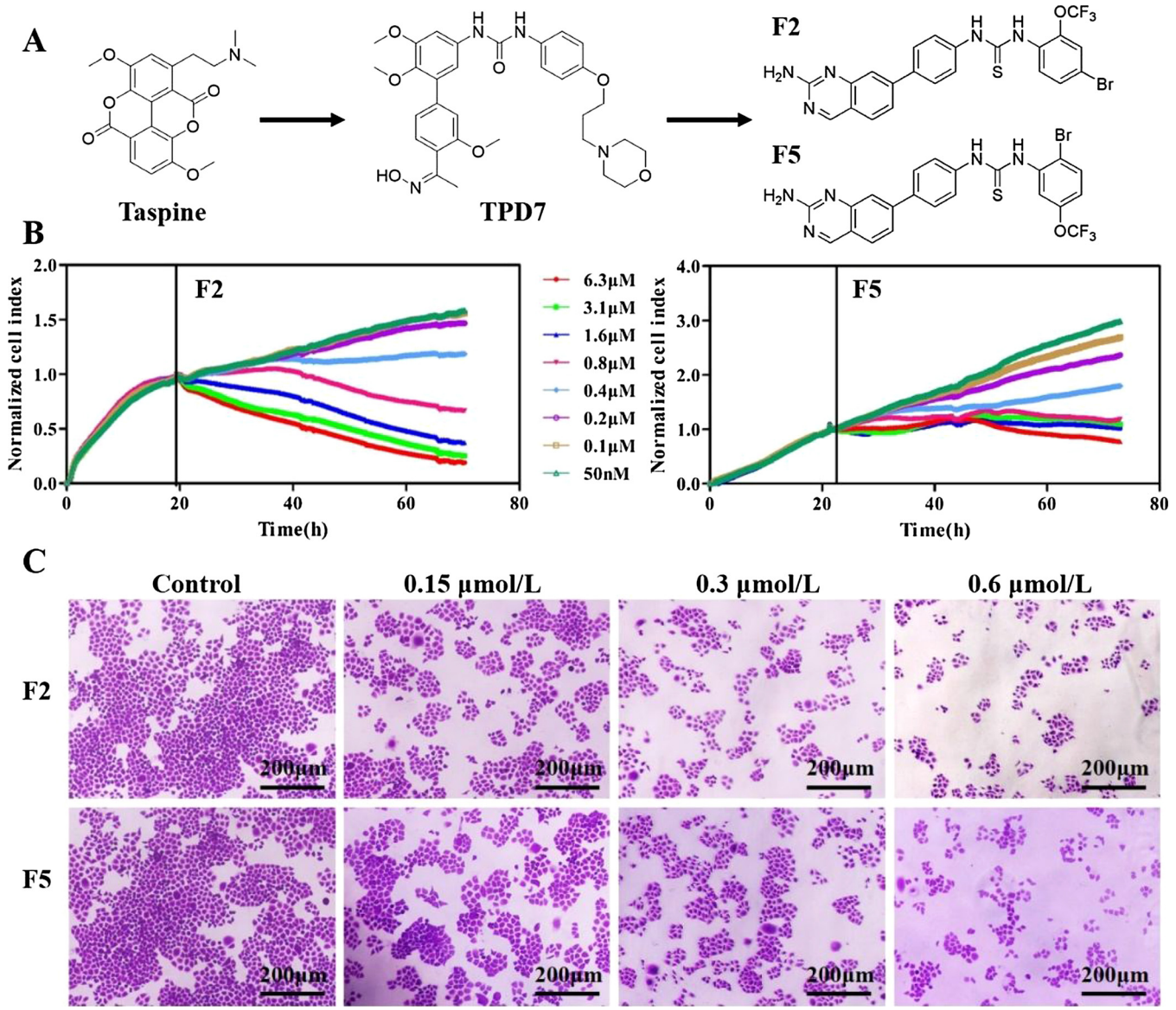

Fig. 1. F2 and F5 inhibited HeLa cells proliferation. (A) The design route of F2 and F5. (B) Real time cell growth curve of F2 and F5 on viability of HeLa cells. Cells were cultured with or without F2 and F5 at indicated concentrations for 48 h. (C) Photographs of HeLa cells stained with 0.2% crystal violet with or without F2 and F5.

the USA were reported to overexpress Stat3 according to a previous study [12], so preventing Stat3 might have great potential for the treatment of the two gynecological cancers.

The feasibility of kinases as therapeutic drugs target have been confirmed, along with countless studies linking JAK/Stat to malignancies, a number of jakinibs including ruxolitinib, tofacitinib and oclacitinib have been approved by FDA [13]. Discovery of active ingredients from natural products and structural optimization is of great importance for the creation of new drugs. Taspine is a kind of apanthrene alkaloids which is isolated from *Radix et Rhizoma Leonticsi* [14]. In the present study, we designed and synthesized two novel isomers compounds TAD-1822-7-F2 (F2) and TAD-1822-7-F5 (F5) from the chemical structure of taspine and its derivatives TPD7 (Fig. 1A). In the present study, we investigated the potential targets of F2 and F5 for their inhibition on HeLa cells proliferation, and explored the underlying mechanism of F2 and F5 as a novel inhibitor of the JAK/Stat signaling pathway.

2. Materials and methods

2.1. Chemicals and reagents

F2 and F5 (purity > 98%) were synthesized in the Research and Engineering Center for Natural Medicine, Xi'an Jiaotong University. AZD1480 was obtained from TargetMol (Massachusetts, USA) and OSM was purchased from R&D system (Minnesota, USA). Human cervical cancer cell HeLa (TCHu187) was purchased from Shanghai Institute of Cell Biology in the Chinese Academy of Sciences (Shanghai, China). Roswell Park Memorial Institute 1640 medium was purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Excell Bio (Shanghai, China). Trypsin was obtained from Amresco (Solon, OH, USA). The penicillin was purchased from General Pharmaceutical Factory (Haerbin, China), and the streptomycin was purchased from North China Pharmaceutical (Shijiazhuang, China). Crystal violet was purchased from Beijing Chemical Plant (Beijing, China). Propidium iodide (PI) was purchased from Sigma-Aldrich. Rabbit anti-GAPDH, Rabbit anti-β-actin, goat anti rabbit IgG,