Int J Clin Exp Med 2015;8(4):6563-6567
www.ijcem.com /ISSN:1940-5901/IJCEM0006626

Original Article

Effectiveness of inhibitor rapamycin, saracatinib, linsitinib and JNJ-38877605 against human prostate cancer cells

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Received February 2, 2015; Accepted April 3, 2015; Epub April 15, 2015; Published April 30, 2015

Abstract: To investigate the effect of different concentrations of inhibitors rapamycin, saracatinib, linsitinib and JNJ-38877605 on PC-3 cells with CCK-8 assay, respectively. PC-3 cells were incubated with different concentrations of rapamycin, saracatinib, linsitinib and JNJ-38877605, respectively, for 48 h at 37°C, the concentrations of rapamycin were 5 nM, 10 nM, 20 nM, 50 nM, 75 nM, 100 nM; Saracatinib: 0.125 nM, 0.25 nM, 0.5 nM, 1 nM, 2.5 nM, 5 nM; Linsitinib: 2 nM, 5 nM, 10 nM, 20 nM, 40 nM, 60 nM; JNJ-38877605: 0.125 nM, 0.5 nM, 1 nM, 2.5 nM, 5 nM, 10 nM. The proliferation of PC-3 cells was examined by CCK-8. Different concentrations of inhibitor rapamycin remarkably inhibited PC-3 cell proliferation after 48 h ($P<0.05$), inhibitory action did not change significantly from 5 nM-100 nM; different concentrations of saracatinib, linsitinib and JNJ-38877605 did not inhibit PC-3 cell proliferation after 48 h. Rapamycin treatment at low concentration can inhibit the proliferation of PC-3 cells, while saracatinib, linsitinib and JNJ-38877605 do not inhibit PC-3 cell proliferation.

Keywords: PC-3 cells, rapamycin, saracatinib, linsitinib, JNJ-38877605

Introduction

Prostate cancer (PCa) is the most commonly diagnosed and the second leading cause of cancer-related mortality in men in the United States, with an estimated 233,000 new diagnoses in 2014 alone and an estimated 29,480 mortalities in 2014 [1]. A great many patients with prostate cancer are treated successfully with androgen deprivation therapy (ADT). Although ever improved androgen ablation therapies prolong life in men with advanced PCa, but most prostate cancer patients will relapse due to the progression of castration-resistant prostate cancer (CRPC) [2]. Therapeutic side effects can be serious [3] and no curative treatment exists after tumors become androgen resistant. Therefore, it is particularly necessary to develop novel therapeutics and diagnostics for androgen resistant prostate cancers and to improve survival of such patients.

Rapamycin is a macrolide antibiotic from Streptomyces hygroscopicus that has been approved by the US FDA as an immunosuppressant and is commonly used to prevent rejection in organ or bone-marrow transplant patients. Rapamycin inhibits the proliferation of transformed cell lines of lymphoid, CNS, hepatic, melanocytic, osteoblastic, myogenic, renal and connective tissue origin, as well as the proliferation of T and B cells transformed by HTLV-1 and EBV, respectively. It was found to be very active against melanoma, ependymoblastoma, mammary and colon tumors [4-7]. Rapamycin inhibits the mammalian target of rapamycin (mTOR) [8], a kinase often upregulated in malignant cells. mTOR is encoded by a single gene FRAP1, is a 289-kDa serine/threonine protein kinase, and controls multiple functions throughout the body that involve gene transcription and protein formation, cytoskeleton composition, metabolism, development, survival, and senescence [9].

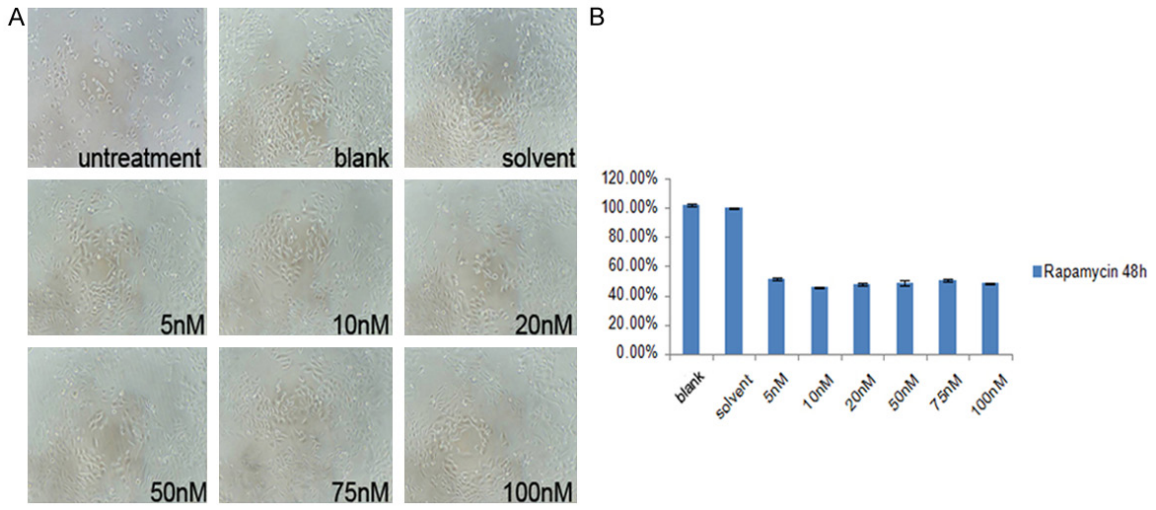

Figure 1. Effectiveness of inhibitor rapamycin against PC-3 human prostate cancer cells. A. Representative photographs from rapamycin treated PC-3 cells after 48 h. B. The proliferation of PC-3 cells treated by rapamycin was examined by CCK-8.

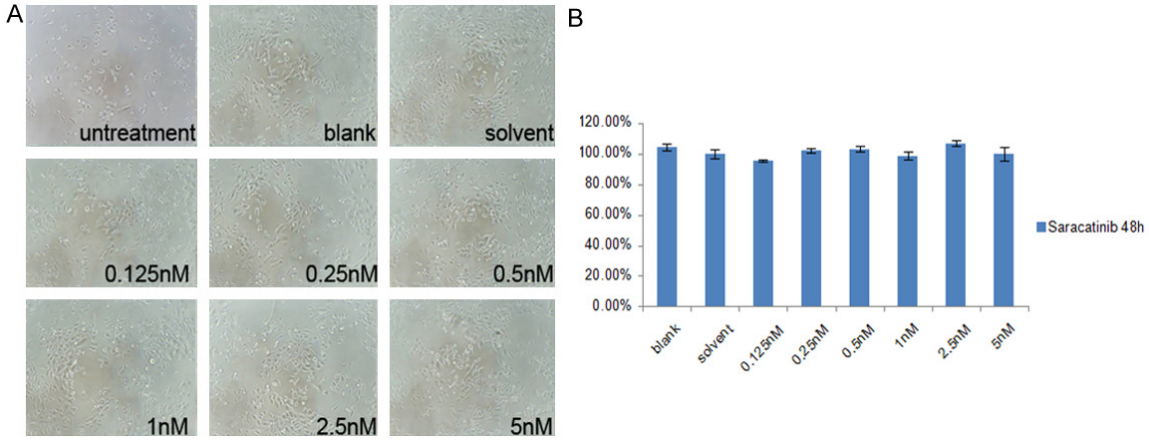

Figure 2. Effectiveness of inhibitor saracatinib against PC-3 human prostate cancer cells. A. Representative photographs from saracatinib treated PC-3 cells after 48 h. B. The proliferation of PC-3 cells treated by saracatinib was examined by CCK-8.

Saracatanib (AZD0530) is an orally available small molecule Src kinase inhibitor that is highly selective for non-receptor tyrosine kinases [10]. It is highly selective for non-receptor tyrosine kinases including c-Src, c-Yes, Lck, and Bcr-Abl. In preclinical studies, saracatinib had an antiproliferative effect in a number of human cancer cell lines including colon, breast and NSCLC and some HNSCC [11, 12], predominantly by greater inhibition of cancer cell motility rather than inhibition of cell growth. It also reduced migration of human lung cancer A549 cells, breast cancer MDA-MB-231 cells and NBT-II bladder cancer cells. Saracatinib is a highly selective, dual Src/Abl kinase inhibitor.

Linsitinib (OSI-906) is a novel, highly selective and orally bioavailable dual insulin-like growth factor 1 (IGF-1R)/insulin receptor (IR) kinase inhibitor with a favorable preclinical profile [13]. A phase III clinical study of linsitinib in patients with locally advanced or metastatic adrenocortical carcinoma is currently being conducted, along with several phase II clinical studies [14, 15]. The MET kinase inhibitor JNJ-38877605 displays selectivity and inhibitory activity against MET.

Given the poor outlook for patients with androgen refractory prostate cancers, we investigate the effect of rapamycin, saracatinib, linsitinib,

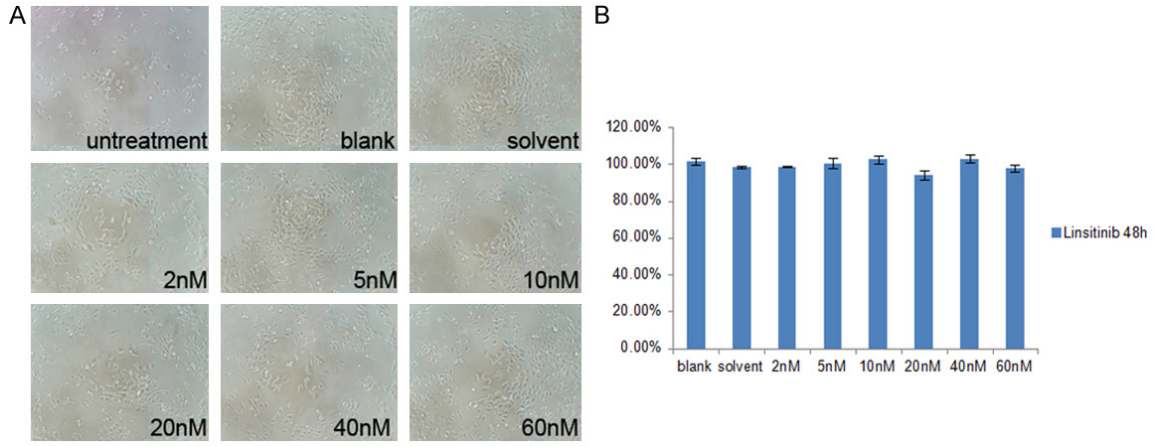

Figure 3. Effectiveness of inhibitor linsitinib against PC-3 human prostate cancer cells. A. Representative photographs from linsitinib treated PC-3 cells after 48 h. B. The proliferation of PC-3 cells treated by linsitinib was examined by CCK-8.

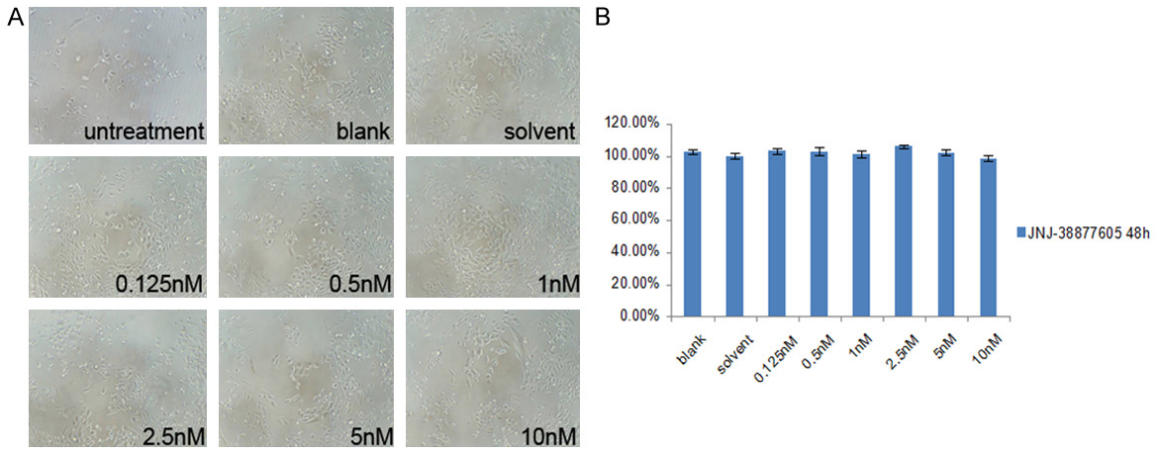

Figure 4. Effectiveness of inhibitor JNJ-38877605 against PC-3 human prostate cancer cells. A. Representative photographs from JNJ-38877605 drug treated PC-3 cells after 48 h. B. The proliferation of PC-3 cells treated by JNJ-38877605 was examined by CCK-8.

JNJ-38877605 on hormone-resistant human prostate cancer cell PC-3, respectively. We conducted the study to evaluate the efficacy and safety of the four different kinds of inhibitors in PC-3 cells in vitro.

Materials and methods

Cell culture and treatment

Hormone-resistant human prostate cancer cells PC-3 were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China), were routinely cultured in RPMI-1640 media (Hyclone Logan, UT). All cultures were supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY) and incubated at 37°C in a 5% CO_2 incubator. Rapamycin: S1039, Saracatinib: S1006, Linsitinib: S1091, JNJ-38877605: S1114 were purchased from Selleckchem. Rapamycin, saracatinib, linsitinib and JNJ-38877605 were diluted in DMSO at a concentration of 2 micromole per liter (mM) as stock solution, respectively, and PC-3 cells were treated with various concentrations of rapamycin, saracatinib, linsitinib and JNJ-38877605.

Cell viability assay

The cell viability and cytotoxicity were detected by Cell Counting Kit-8 (Dojindo, CK04). Cells (4×104/ml) were plated in 96-well plates at a total volume of 100 μl per well and photo-

graphed before being treated with inhibitors. Cells were treated with various concentrations of rapamycin (5 nM, 10 nM, 20 nM, 50 nM, 75 nM, 100 nM), saracatinib (0.125 nM, 0.25 nM, 0.5 nM, 1 nM, 2.5 nM, 5 nM), linsitinib (2 nM, 5 nM, 10 nM, 20 nM, 40 nM, 60 nM), JNJ-38877605 (0.125 nM, 0.5 nM, 1 nM, 2.5 nM, 5 nM, 10 nM) for 48 h at 37°C, respectively, then photographed. 10 μl of CCK-8 solution was added to each well after 48 h incubation and cultivated for another 3 h at 37°C, and then the OD value for each well was read at wavelength 450 nm to determine the cell viability on a microplate reader (Thermo, MuLTiSKAN MK3). Cell viability was calculated with the formula: percent viability (%) = [OD (drug-treated sample) -OD(blank)]/[OD (untreated sample) -OD (blank)] ×100%.

Statistical analysis

All experiments were repeated at least three times and data analysis was performed by using Microsoft Excel software. Values were presented as means ± standard deviation (SD). Statistical differences were performed with one-way analysis of variance (ANOVA) by the statistical software SPSS 18.0. $P<0.05$ was considered to be statistically significant.

Results

The states of the PC-3 cells were almost identical before being treated with inhibitors, respectively. As shown in **Figure 1**, different concentrations of inhibitor rapamycin remarkably inhibited PC-3 cells proliferation after 48 h ($P<0.05$), low concentrations of rapamycin inhibited PC-3 cells proliferation evidently after 48 h, the inhibitory action did not change significantly from 5 nM-100 nM. However, different concentrations of saracatinib, linsitinib and JNJ-38877605 did not inhibit PC-3 cells proliferation after 48 h, high or low concentrations of the three inhibitors did not inhibit PC-3 cells proliferation at all (**Figures 2**-**4**).

Discussion

Prostate cancer is one of the most common solid neoplasms and recognized as one of the most important medical problems among the male population. Most prostate cancer-related deaths are due to metastasis [16]. Prostate cancer is an ideal target tumor for chemoprevention, because of its long latency and identifiable preneoplastic lesions. Chemotherapy is the typical treatment for patients with advanced stage of PCa; however, it is usually ineffective in hormone-resistant human prostate cancer cells.

The results of this study show that in PC-3 cells, rapamycin strongly inhibits cells proliferation, while saracatinib, linsitinib and JNJ-38877605 cannot inhibit PC-3 cells proliferation. Rapamycin, having been used for years to prevent rejection in kidney transplant recipients, is a negative regulator specific to mTOR signaling pathway [17]. Recent reports have shown that rapamycin can ameliorate kidney fibrosis by inhibiting the activation of mTOR signaling pathway in interstitial macrophages and myofibroblasts and inhibit the formation of renal cysts with polycystic kidney disease in mice [18]. A study has also shown that rapamycin can inhibit acute myeloid leukemia cells by inducing a blockade at the G0/1 phase of the cell cycle via the inhibition of mTOR signaling [19]. Saracatinib is a highly selective, dual Src/Abl kinase inhibitor. In a preclinical study [11], saracatinib exerted potent anticancer effect in vitro and inhibited tumor metastasis in vivo, but it did not inhibit PC-3 cells proliferation.

In the future study, more work is needed to investigate how rapamycin is involved in the maintenance of mTOR signal pathway and the autophage in vivo and in vitro, to define their roles in different pathologic processes of PC-3 cells. These findings indicate that rapamycin may have relevant implications in the prevention and treatment of this malignancy.

Acknowledgements

This work was supported by Scientific Research Funds from Shandong Provincial Medicine and Health Science and Technology Development Plan (2014WS0284), and Linyi Municipal Science and Technology Development Project (201413015).

Disclosure of conflict of interest

None.

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