Hsp90 inhibitor SNX-2112 induce autophagy and apoptosis in MDA-MB-468 cells

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Abstract

Breast cancer threatens people health. In the word, it is the most common hematologic malignancy, and ranks within the top 10 of common malignant tumors in annual year. Heat shock protein 90 (Hsp90) is high expression level in cancer cells, which is an ATP-dependent protein that can interact lots of other proteins. Hsp90 plays an important role in signal transduction pathways which are regulated in cell proliferation, differentiation, and migration. SNX-2112, a novel Hsp90 selective inhibitor on proliferation of breast cancer cells. However, the anticancer effect of inhibiting Hsp90 has not been currently unknown, so the anticancer mechanisms is needed to deeply identify. In this study, we investigated the inhibitory and regulatory mechanisms of SNX-2112, a novel Hsp90 selective inhibitor on proliferation of MDA-MB-468 cells. SNX-2112 induced apoptosis in MDA-MB-468 cell line, as shown by the activation of caspases and cleavage of Poly (ADP- ribose) polymerase and downregulation of Bcl-2 and Bcl-xl. The ability of SNX-2112 to induce autophagy was verified by accumulation of beclin-1, and decrease the expression level of proteins in Akt/mTOR signaling pathway, such as p-Akt, p-mTOR and p-4EBP1 in MDA-MB-468 cell line. Together, these results support important evidences to explain the SNX-2112 anticancer activity and and may be a promosing drug of breast cancer.

Keywords

Breast cancer; MDA-MB-468 cells; SNX-2112; apoptosis; autophagy.

1. Introduction

Breast cancer is not a single disease but an umbrella for a group of independent diseases which with strong heterogeneity. In our country, it is the most common hematologic malignancy, and ranks within the top 10 of common malignant tumors [1]. At present, in female patients, breast cancer occurs most commonly [2]. In clinical, breast cancer is a diverse group of haematological malignancies, classified luminal A, luminal B, human EGF 2 and basal-like tumors and expresses characteristic proteins,such as ER, PR, HER2 [3]. Breast cancer is also easily circulated to the whole body. Cancer treatments are complicated, especially for breast cancer. At present, the treatments for breast cancer include chemotherapy (such as Cyclophosphamide, Adriamycin, Vincristine, Prednisone, *etc*), hematopoietic stem cell transplantation and targeted new drug therapy [4]. Nevertheless, traditional treatment still is the major therapy, the effect is unsatisfied, new drug is urgent to find [5].

Mitochondria possesses central function in eukaryotic cells, and the abnormal mitochondria proteins are related to lots of diseases [6]. It has reported that the abnormal nuclear-encoded mitochondrial genes bring about the expression level of mitochondria proteins, which lead to the cell physiology change [7].

Heat shock protein 90,which plays a major role in cell physiology, is an ATP-dependent protein[8].Hsp90 plays a major role in proteins processes of folding, assembly and degradation[9,

10].And Hsp90 regulates the client proteins, such as Akt, IKK and Erk [11].However, these client protein, which involve cell apoptosis and survival ,decide generation and development of cancer[12, 13]. A lot of studies have showed that the Hsp90 expression level in tumor tissues is 2-10 fold with normal tissues, which is important for tumor progression[14].It will be an attractive therapeutic treatment for cancer by suppressing Hsp90.

SNX-2112 selectively suppresses Hsp90 by binding competitively to the Hsp90 N-terminal, and also showed anticancer activity against some kinds of tumors, including breast cancer [15, 16]. Moreover, SNX-2112 possesses better treatment effect than others [17].But the effect and the molecular mechanism of SNX-2112 on breast cancer cells are known and need further explore. In this study, these results suggest that SNX-2112 can suppress cell proliferation in breast cancer MDA-MB-468 cells. In this study, it reports SNX-2112 decreases the expression levels of proteins, such as Bcl-2, Bcl-X_L, p62, p-p65, p-IKBα, p-Akt, p-mTOR and p-4EBP1 in Akt/mTOR signaling pathway, and upregulate expression levels of cleaved-caspase3, cleaved-caspase7, cleaved-PARP and beclin-1.

2. Materials and methods

2.1 Reagent

All reagents and chemicals were obtained from standard commercial sources. Antibodies against the phosphorylated forms of Akt, mTOR,4EBP1,p65,IκBα;4EBP1; cleaved- Caspase3; cleaved-Caspase7 ; cleaved-PARP Bcl-2; Bcl-X_L; β-actin; SQSTM1/P62; Beclin-1 were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-mouse IgG and anti-rabbit IgG were purchased from Sigma (St. Louis, MO, USA).Cell Counting Kit-8 (CCK-8 Kit) was purchased from Dojindo (Shanghai, China).

2.2 Cell culture

Breast cancer MDA-MB-468 cells was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China); Breast cancer MDA-MB-468 cells were cultured in suspension in DMEM (Gibco, Thermo Scientific, Waltham, MA, USA) completed medium which containing 10% fetal bovine serum (FBS, Gibco), 10 mg/mL streptomycin and 100 U/mL penicillin at 37 ℃ in a humidified atmosphere containing 5% CO_2.

2.3 Cell Proliferation Analysis

The proliferation inhibition of MDA-MB-468 was evaluated using CCK-8 kit after treated with different concentrations of SNX-2112. Briefly, 100 μL suspension containing 4000 MDA-MB-468 cells which in the logarithmic growth phase were plated into 96-well plates per well and were treated with a range of SNX-2112 concentrations. Then cells were continuously cultured at 37 ℃ with 5% CO_2. After 48 h, 10 μL of CCK-8 reagent was added to each well, respectively and incubated for 2 h at 37 ℃. Finally, the optical density at 450 nm was detected using a Microplate Reader.

2.4 Western blot

After treated with 0, 1, 5, 10 μM SNX-2112 for 48 h (see above), MDA-MB-468 cells in each group were harvested, washed with ice-cold PBS, and lysed with RIPA buffer containing 0.1 M PMSF, 0.5 M DTT, 20× protease inhibitor cocktail, and 20× phosphatase inhibitor cocktail cracked by ultrasonication, and centrifuged at 12,000 rpm for 15 min., the super natants were collected by centrifugation. Then the protein was denatured by boiling and detected the concentration by BCA Protein Assay reagent kit. The protein was fractionated using SDS-PAGE and transferred onto PVDF membranes(Millipore, USA), and membranes were blocked for 1 h in 5% skim milk before being incubated with antibodies. For detection, the membrane were incubated at 4 ℃ overnight with primary antibodies and washed 3 times in TBST, each time for 10 min. Then the membrane was incubated with secondary antibodies for 1 h at room temperature. Finally, the membrane was washed with TBST repeatedly again and the protein was visualized using the ECL kit and observed by GeneGnome mechine (Syngene).

3. Results

3.1 SNX-2112 significantly inhibits the cell viability of MDA-MB-468 cells

The chemical structures of SNX-2112 were illustrated in figure.1 to investigate the anti-tumor activities of these compounds in *vitro*, CCK-8 assay was used to examine the effects of SNX-2112 on the cell viability of MDA-MB-468 cells in figure.2. As shown in figure.2, SNX-2112 could effectively inhibit MDA-MB-468 cells viabilities. Moreover, the inhibitory effect on MDA-MB-468 cells also increased significantly as the treated concentration of SNX-2112 increasing. And the IC50 value of the most potent compound for MDA-MB-468 cells was 0.76 μM. All these results suggested SNX-2112 may has an effectively anti-tumor ability.

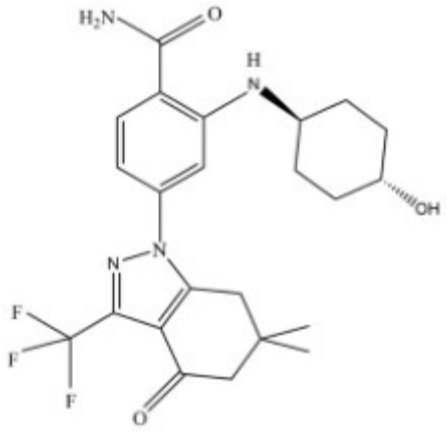

Fig. 1 Chemical structure of SNX-2112

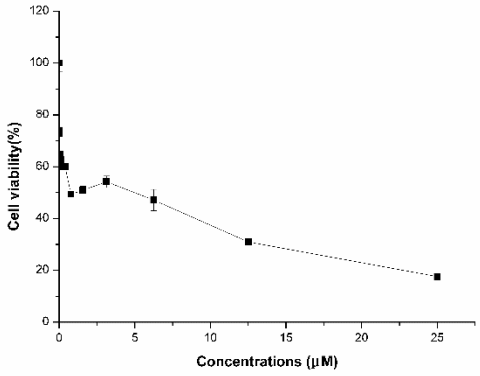

Fig. 2 Effect of SNX-2112 on proliferation of breast cancer cell. Cells (4×10^4 cells/mL) were treated with a range of concentrations of SNX-2112 (0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5 and 25 μM) for 48 h. The CCK-8 assay was used to detect the cell viability of MDA-MB-468 cells.

3.2 SNX-2112 induces the apoptosis of MDA-MB-468 cells

As is well known, the reasons which caused cell death are mainly necrosis, apoptosis, and autophagy, and most chemotherapy drugs used in tumor therapy exert their efficacy via inducing cell apoptosis. Hence, we detected apoptosis changes of MDA-MB-468 cells after treated with different concentrations of SNX-2112 using western blot firstly. As is shown in figure3 and figure4 , SNX-2112 was able of inducing apoptosis in MDA-MB-468 cell line, upregulating caspase signaling proteins (Caspase-3 and -7), activating the downstream death substrate PARP, but downregulating expression levels of anti-apoptotic proteins Bcl-2 and Bcl-X_L. These results indicate that SNX-2112 induce the apoptosis in MDA-MB-468 cells.

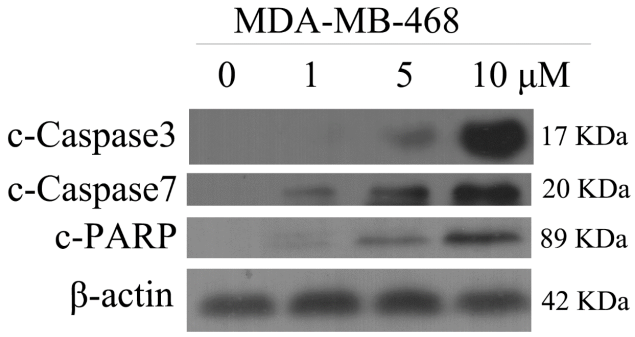

Fig. 3 SNX-2112 induces caspase activation in MDA-MB-468 cells

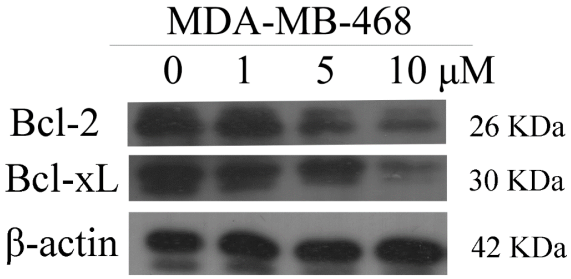

Fig. 4 Western blot analysed the effect of SNX-2112 on the expression of Bcl-2 family proteins in MDA-MB-468 cells

3.3 SNX-2112 induces autophagy in MDA-MB-468 cells

As shown in figure 5， SQSTM1/P62 generation decreases, which is an autophagy protein, Beclin-1 is a key protein in cell autophagy, and beclin-1 production increases under the influence of SNX-2112.These results demonstrate that SNX-2112 could induce MDA-MB-468 cells autophagy.

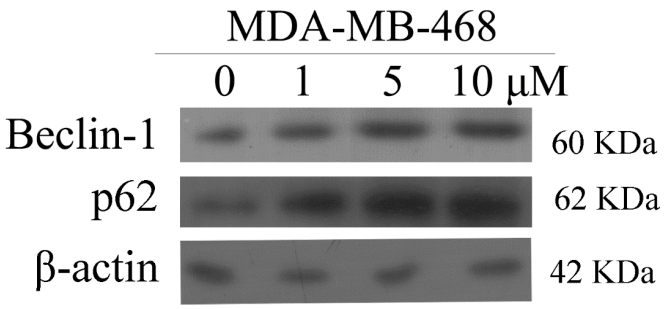

Fig. 5 Western blots were used to measure the levels of autophagy markers: Beclin-1 and SQSTM1/p62

3.4 SNX-2112 regulates autophagy through the Akt/mTOR signal transduction pathway.

The Akt/mTOR signal transduction pathway has been revealed it influences cell autophagy. MDA-MB-468 cells were treated with different concenstrations of SNX-2112 for 48 h.As shown in as is shown in figure6. Akt phosphorylation level decreased after SNX-2112 treatment for 48 h, indicating the regulation of Akt at SNX-2112-induced autophagy. In addition, SNX-2112 inhibits p- Akt level, the downstream protein of p- mTOR, the p-4EBP1.Western blot revealed that SNX-2112 treatment of MDA-MB-468 didn’t inhibit nonphosphorylated proteins. These results suggest that PI3K/Akt signaling pathway is involved in SNX-2112 induced autophagy in MDA-MB-468 cells.

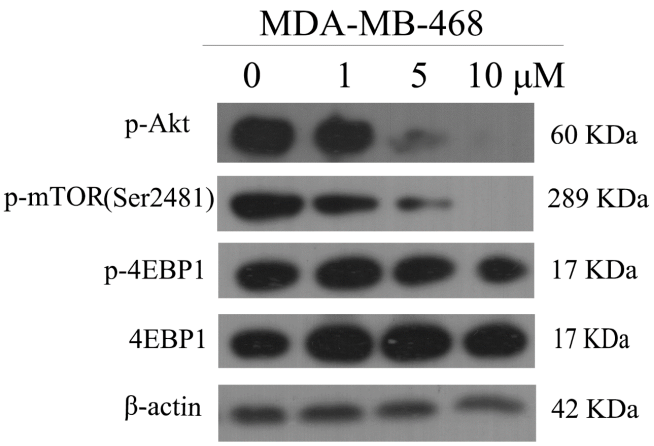

Fig. 6 SNX-2112 downregulates the Akt/mTOR pathway-associated proteins to induces autophagy in MDA-MB-468 cells

3.5 SNX-2112 Supresses NF-kB pathway Activation

Hsp90 is considered as a major role for cancer cell growth and proliferation, NF-kB pathway through Hsp90, which depend IKK activation is constitutively activated. The results show that SNX-2112 induced inhibition of p- IκBα, p-p65.For NF-kB, it must translocate into nucleus to regulate gene transcription. To determine if p65 accumulation in the nucleus, we detect the influence of SNX-2112 on NF-kB activation by western blot. As is shown in figure7, p65 should translate into the nucleus to activate antiapoptosis gene transcription. These results suggest that SNX-2112 inhibits NF-kB activation.

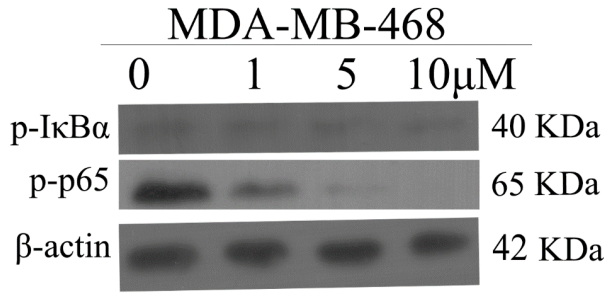

Fig. 7 Western blot analysed the effect of SNX-2112 on the expression of NF-κB signaling pathway-related proteins in MDA-MB-468 cells

4. Discussion

Breast cancer is high universality in female patients. Traditional treatment not only have a good effect, but also have side effects on cancer patients. Chemotherapy, the main approach for the treatment on tumors, has earned a good effect[18]. At present, lots of studies have revealed that Hsp90 expression level is high in tumor tissues, which reveals Hsp90 is a covert therapeutic target against cancer[19]. SNX-2112, a selective small inhibitor of Hsp90, has anti-tumor activity against breast cancer [20].

In this study, we verified the anti-cancer activity of SNX-2112 by CCK-8 assay. The result indicated that SNX-2112 could effectively inhibit the cell viability of breast cancer cells. Some studies suggest that the cell viability is related to apoptosis, which bases on the degradation of cellular proteins by a group of caspases [21, 22]. Moreover, SNX-2112 was able of inducing apoptosis in MDA-MB-468 cell line, upregulating caspase signaling proteins (Caspase-3 and -7), activating the downstream death substrate PARP, but downregulating expression levels of anti-apoptotic proteins Bcl-2 and Bcl-X_L. These results indicate that SNX-2112 induce the apoptosis in MDA-MB-468 cells.

Autophagy is a component of the cell that is degraded by lysosomes. Accordingly, cytoplasmic component is isolated within autophagosomes and is merged in the lysosomal compartment. We investigated the effect of SNX-2112 on autophagy of MDA-MB-468 cells through different methods

and the expression level of SQSTM1/P62 decreased and beclin-1 increased as the concentration of SNX-2112 increased.

In cancer cells, PI3K induces cell apotosis,autophagy, and metabolism[23].This signal transduction pathway contains signal mediator Akt and mTOR [23]. Akt/mTOR/4EBP1 signal transduction pathway has been reported to downregulate the autophagy[24].In this study, we disclosed the mediators in Akt/mTOR/4EBP1 signal pathway, such as p-Akt,p-mTOR, and p-4EBP1,the expression levels of them decreased in a dose-dependent manner after SNX-2112 treatment. These results show that the Akt/mTOR/4EBP1 signal pathway implicated in SNX-2112-induced autophagy, and upregulates apoptosis in breast cancer cells. Akt could provide a positive effect on NF-kB pathway. NF-κB subunit can bind DNA cooperatively and activate transcription with other heterologous transcription factors. The inhibition of NF-kB pathway by downregulating p-p65 and p-IKBαexpression level.

5. Clnclusion

In summary, The results showed that SNX-2112 supressed better anticancer activity against MDA-MB-468 cells. It confirmed that SNX-2112 could effectively supress the cell proliferation in breast cancer MDA-MB-468 cells. Taken above results, we finally suggested that SNX-2112 inhibited the cell proliferation of breast cancer cells via mediating expression of Bcl-2 familiy proteins, NF-kB pathway, Akt/mTOR signal transduction pathway and caspase signaling proteins. This study shown that SNX-2112 had a future for breast cancer therapy.

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