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14. ABSTRACT

The present study demonstrated SNCG can partially replace the chaperoning function of Hsp90 and protect HER2/Akt/mTOR stability and function in the stressful condition when the function of Hsp90 is blocked. Disruption of Hsp90 with 17-AAG resulted in a significant degradation of HER2. However, expression of SNCG completely recovered 17-AAG-mediated losses of HER2. This SNCG-mediated protection was demonstrated in breast cancer cells, tumor xenograft, and mammary glands from HER2/SNCG bitransgenic mouse. Consistent with its chaperoning activity, SNCG bound to HER2 in the presence and absence of Hsp90. While expression of SNCG renders resistance to 17-AAG-induced apoptosis both in vitro and in tumor xenograft, knockdown endogenous SNCG enhances the sensitivity to the Hsp90 disruption. Crossing SNCG transgenic mice with HER2 mice stimulated HER2-induced tumor growth and rendered resistance to 17-AAG-mediated antitumor effect on the transgenic mice. The present study indicates that SNCG functions as a tumor specific chaperone, which can replace the chaperoning function of Hsp90, protect its client protein HER2, and render a resistance to Hsp90 disruption.

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INTRODUCTION

A no-cost extension was granted last year to extend the final budget period through July 31, 2009. In this extended one year period, we generated some exciting additional data (not proposed in the original grant) on SNCG and HER2 function: 1) Expression of SNCG in mammary gland exacerbates HER2-induced mammary tumor development. 2) SNCG physically interacts and transactivates HER2 in breast cancer cells. 3) SNCG prevents induced HER2 degradation. We will test a new hypothesis that SNCG chaperones HER2 by stimulating HER2 signaling and preventing its degradation, and thus renders HER2 overexpressing breast cancer cells resistant to Herceptin-based therapy.

HER2-positive tumors account for approximately 30% of all breast cancer and these tumors carry poor prognosis. The importance of HER2 in breast cancer led to the development of agents that aimed at reducing HER2 level or activity. Trastuzumab (Herceptin) and lapatinib are 2 agents that have gained FDA approval for treating HER2-positive breast cancer. In spite of their impressive results, a significant fraction of patients still develop either primary or secondary resistance. Several possible mechanisms of resistance have been proposed and one of the most described mechanisms is the loss of PTEN activity and/or activation of PI3K/Akt/mTOR signaling pathway. In addition, biomarkers including p95HER2, IGF1R, and others have been linked to resistance to HER2-targeting agents (1) Hsp90 is a ubiquitously expressed molecular chaperone that controls the folding, assembly, intracellular disposition, and proteolytic turnover of many proteins, most of which are involved in signal transduction processes (2-4) Hsp90 keeps unstable proteins poised for activation until they are stabilized by conformational changes associated with signal transduction (5-6). Considering that HER2 is an Hsp90 client protein and requires interaction with Hsp90 and its chaperone to acquire proper protein function (7), the Hsp90 inhibitor such as geldanamycin provides an alternative approach to target HER2 through dissociation of HER2 from the chaperone, which leads to HER2 degradation by a proteasome-dependent manner (8). Currently, the less toxic analogue of geldanamycin, 17-AAG, is being actively evaluated in multiple phase II/phase III clinical trials. Our previous studies demonstrate that expression of SNCG was strongly correlated with lymph node involvement, metastasis, and HER2 status (9). In the present study, we evaluated the effect of SNCG on HER2 expression and function and on Akt/mTOR signaling pathway. The results indicate that SNCG has chaperoning activity that protects HER2 and Akt/mTOR stability and function under stressful conditions when the chaperoning activity of Hsp90 is blocked.

BODY

SNCG prevents Hsp90 inhibitor 17-AAG induced HER2 degradation. Hsp90 is a molecular chaperone whose association is required for the stability and function of multiple signaling proteins. Hsp90 inhibitor 17-AAG binds to the ATP-binding domain of Hsp90 and blocks its activity, resulting in the dissociation and eventual degradation of Hsp90-interacting proteins (10). Clinical follow-up studies indicate that expression of SNCG in breast cancer renders resistance to adjuvant therapy (9). These clinical studies also demonstrated that expression of SNCG is clinically associated with HER2 status (9). Since the stability and function of HER2 is regulated by Hsp90 and 17-AAG acts primarily by promoting the degradation of HER2 (11), we investigated whether expression of SNCG could prevent 17-AAG-induced HER2 degradation. Using previously established SNCG transfected MCF-7 cell (12-13), we demonstrated that while treatment of parental MCF-7 and vector transfected MCF-neo1 cells with 17-AAG resulted in a significant decrease in HER2 expression; forced expression of SNCG completely prevented the induced HER2 degradation (**Fig. 1A**). Treatment of the cells with Herceptin had no effect on HER2 expression levels. Protein degradation in cells can be achieved by several protease systems; however, most proteins, the stability of which is regulated by

Hsp90, appear to be degraded by proteasomes (10). To investigate whether the reduction of HER2 was due to proteasomal degradation, we treated MCF-7 cells with proteasome inhibitor MG132 to prevent the 17-AAG-induced degradation of HER2. The results of this treatment revealed that 17-AAG-induced HER2 degradation can be blocked by proteasome inhibitor (**Fig. 1B**). These results indicate that dysfunction of Hsp90 by treatment with Hsp90 inhibitors destabilized the HER2 protein and the unstable HER2 was removed by proteasomes.

The effect of SNCG expression on 17-AAG-induced HER2 degradation was further demonstrated by inhibiting endogenous SNCG expression in T47D cells using previously established stable SNCG knockdown T47D cell lines: AS-3 cells (12). As demonstrated in **Fig. 1C**, knockdown SNCG in T47D cells significantly increased sensitivity to 17-AAG-induced HER2 degradation. Since SNCG activates ERα transcriptional activity (13) and ERα and HER2 cross activate each other, it is likely that the observed SNCG-mediated HER2 protection in ER-positive MCF-7 and T47D cells is mediated indirectly by stimulation of ERα activity. To exclude this possibility, we first used ER-negative and SNCG stably transfected MDA-MB-435 cells (**Fig. 1D**). As we demonstrated in ER-positive MCF-7 cells, 17-AAG treatment caused a significant reduction of HER2 levels in parental SNCG-negative MDA-MB-435 cells and control vector transfected neo-435-1 cells. However, this 17-AAG-induced downregulation of HER2 was completely blocked by expression of SNCG in stably transfected SNCG-435-3 cells. Since there has been a controversy over the past several years about the true origin of the human MDA-MB-435 cell line, which might be derived from M14 melanoma cells (14), next we used ER-negative and SNCG-positive SKBR3 breast cancer cell, which is considered as HER2 over-expressed cell line. The effect of SNCG knockdown on HER2 expression in response to 17-AAG treatment was determined in SKBR3 cells (**Fig. 1E**). Treatment of SNCG knockdown cells with 17-AAG significantly reduced HER2 expression levels. In contrast, expression of endogenous SNCG partially prevented 17-AAG-induced HER2 degradation. These data suggest that SNCG prevents HER2 degradation under stressful conditions, in which the chaperone function of Hsp90 is blocked by 17-AAG; and such protection of HER2 is ERα-independent.

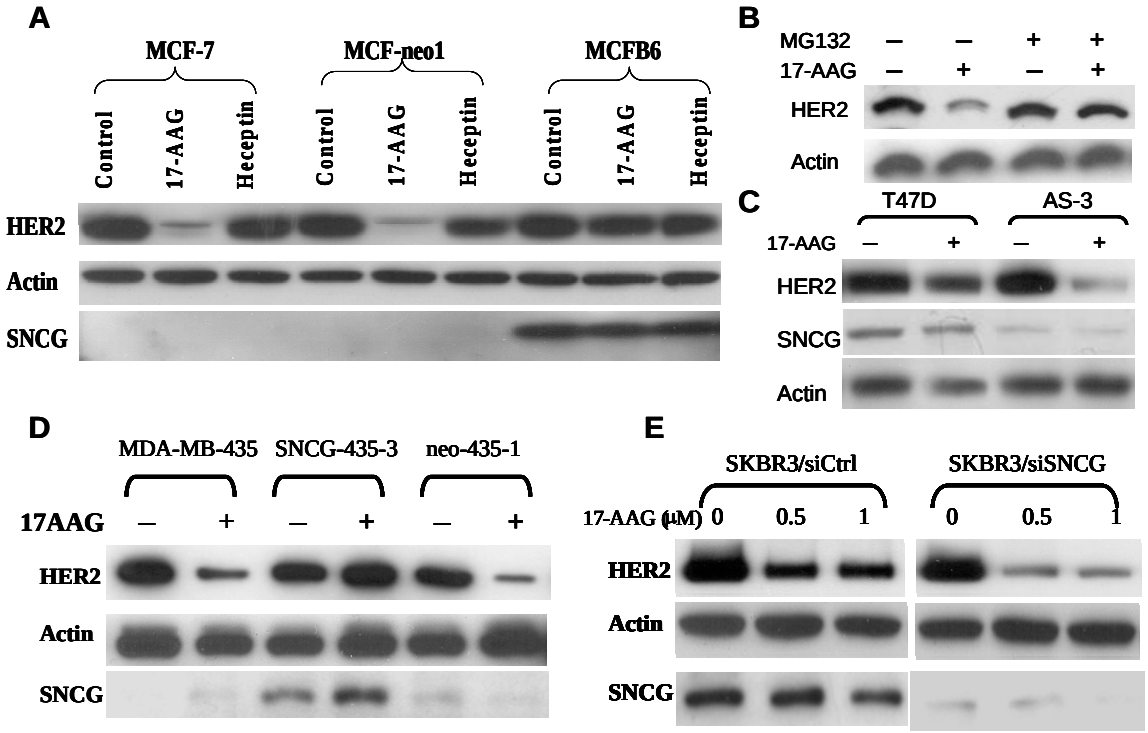
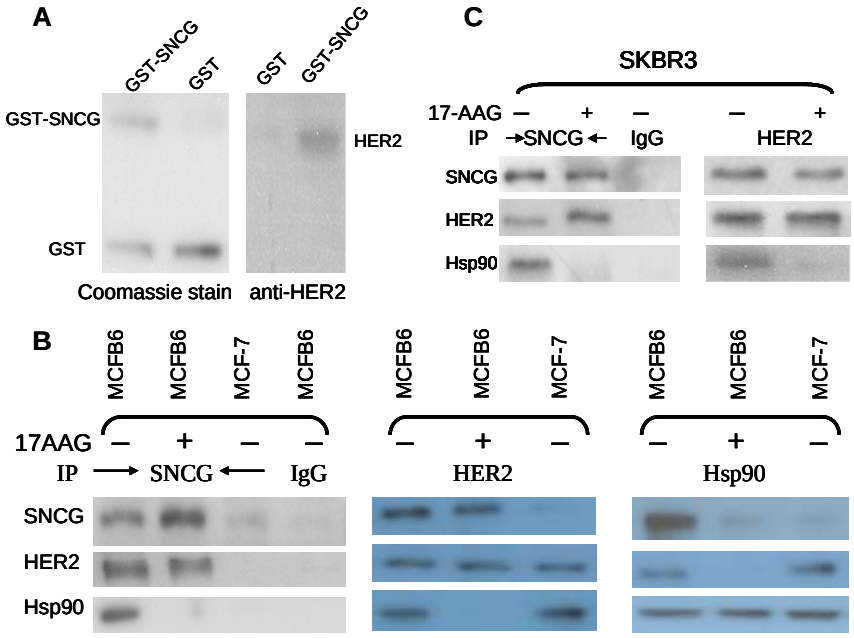


Fig. 1. Prevention of HER2 degradation by SNCG. **A**. MCF-7, MCF-neo1 (vector transfected clone), and SNCG stably transfected MCFB6 cells were treated either with 17-AAG (1 μM) or Herceptin (1 μM) for 12 hours. HER2 and SNCG expression were analyzed by Western blot and normalized with actin. **B**. Reduction of HER2 was prevented by proteasome inhibitor treatment. MCF-7 cells were treated 17-AAG (1 μM) for 10 h after pretreatment with 20 μM of proteasome inhibitor MG-132 for 2 h. Cell lysates were analyzed for HER2 expression. **C**. T47D and its SNCG knockdown AS-3 cells were treated with 17-AAG (1 μM) for 12 hours. HER2 and SNCG expression were analyzed by Western blot **D**. Parental MDA-MB-435, SNCG stably transfected SNCG-435-3, and control neo-435-1 cells were treated with 1 μM 17-AAG for 15-h. **E**. Prevention of HER2 degradation in ER-negative SKBR3 cells. Control siRNA (siCtrl) and SNCG siRNA infected cells treated with 17-AAG (0.5 and 1 μM) for 12 hours. HER2 and SNCG expression were analyzed by Western blot.

SNCG physically interacts with HER2. Previous studies demonstrated that SNCG functions like a chaperone, which physically binds to ERα and stimulates its transactivation (13). Because SNCG prevented 17-AAG-mediated degradation of HER2, we investigated if SNCG physically interacts with HER2. We performed a GST pull-down assay using the purified GST-tagged SNCG protein to pull down Hsp90 (**Fig. 2A**). The GST-tagged SNCG was immobilized to GST beads and incubated with lysates of SKBR3 cells. The eluted proteins were subjected to immunoblot analysis using anti-HER2 antibody. The results of immunoblotting revealed that HER2 was specifically precipitated by immobilized GST-SNCG, indicating that HER2 directly interacts with SNCG *in vitro*.

Using SNCG transfected MCFB6 cells, we also determined if SNCG physically interacts with HER2 in cells in the presence and absence of Hsp90 inhibitor 17-AAG by immunoprecipitation (IP) assays. IP of SNCG in SNCG-positive MCFB6 cells co-precipitated HER2 and Hsp90 in the absence of 17-AAG, indicating that SNCG participated in a chaperone complex with Hsp90 and HER2 in the absence of Hsp90 inhibitor. As negative controls, IP of SNCG in SNCG-negative MCF-7 cells and IP with control IgG in SNCG-positive MCFB6 cells did not pull down HER2. Similarly, IP of HER2 co-precipitated SNCG and Hsp90 and IP of Hsp90 co-precipitated HER2 and SNCG in MCFB6 cells (**Fig. 2B**). As expected, after cells were treated with 17-AAG, Hsp90 dissociated from its client protein HER2. However, although SNCG dissociated from Hsp90, it still bound to HER2. Since SNCG still bound to HER2 even after Hsp90 was dissociated from its client protein, these data indicate that although SNCG participated in the chaperone complex with Hsp90, its function on HER2 was mediated by Hsp90-independent pathways such as by direct binding to and chaperoning HER2. We also investigated the *in vivo* interactions among endogenous SNCG, HER2 and Hsp90 in SKBR3 cells (**Fig. 2C**). The same interaction pattern between endogenous SNCG and HER2 and Hsp90 was observed in SKBR3 cells as that we demonstrated in SNCG transfected MCF-7 cells. When SKBR3 cells were cultured in the absence of 17-AAG, endogenous SNCG was co-precipitated with HER2 and Hsp90. However, after the treatment of 17-AAG, Hsp90 dissociated from HER2 and SNCG, but SNCG still bound to Hsp90,



indicating that the interaction between the endogenous SNCG, HER2, and Hsp90 proteins also occurs in the physiological situation. Taken together, these data suggest that at the stressful condition when the function of Hsp90 is disrupted, SNCG functions as a chaperone protein, which physically binds to HER2 and protects its stability.

Fig. 2. SNCG interacts with HER2. **A**. GST-SNCG fusion protein was expressed in *E. coli*, purified, and stained with Coomassie Blue to demonstrate the expression of the fusion proteins (A, left panels). The SKBR3 cell extracts were subsequently incubated either with bead-bound GST as a negative control or GST-SNCG. After the beads were washed, proteins were subjected to Western blot for HER2 (A, right panel). **B**. Parental MCF-7 and SNCG transfected MCFB6 cells were treated with or without 1 μM 17-AAG for 15 hours. Equal amount of protein was subjected to IP with antibodies against SNCG, HER2, and Hsp90 followed by Western blot for SNCG, HER2, and Hsp90. As a negative, IP with control IgG did not poll down SNCG, HER2, and Hsp90 in MCFB6 cells. **C**. SKBR3 cells were treated with or without 1 μM 17-AAG for 15 hours. Equal amount of protein was subjected to IP with antibodies against SNCG and HER2 followed by Western blot for SNCG, HER2, and Hsp90. As a negative, IP with control IgG did not poll down SNCG, HER2, and Hsp90 in SKBR3 cells.

SNCG renders resistance to Hsp90 inhibitor 17-AAG. Inhibition of Hsp90 by 17-AAG leads to degradation of Hsp90 regulated client proteins, inactivation of the corresponding signaling pathways, and apoptosis (10). Since SNCG protects HER2, in the stressful conditions, in which the chaperone activity of Hsp90 is blocked by 17-AAG, we studied the biological relevance of SNCG on rendering resistance in breast cancer cells in response to 17-AAG. As demonstrated in **Fig. 3A**, while treatment of SNCG-negative MDA-MB-435 cells with 17-AAG induced a 55% of apoptotic cells, expression of SNCG rendered cellular resistance to 17AAG, which resulted in only 25% of apoptotic cells. The effect of SNCG expression on 17-AAG resistance was further demonstrated by inhibiting endogenous SNCG expression in SKBR3 cells. SNCG siRNA significantly reduced endogenous SNCG expression in SKBR3 cells. In non-treated cells, there was no significant difference in apoptosis between siSNCG-infected and control siCtrl-infected cells. Treatment of siCtrl-infected control cells with 17-AAG led to a 23% apoptotic cells. This 17-AAG-mediated apoptosis was significantly increased in the SNCG knockdown cells resulting in a 51% of apoptotic cells (**Fig. 3B**). We also analyzed the protective effect of SNCG on 17-AAG-mediated cytotoxicity in an anchorage-independent growth of MDA-MB-435 cells (**Fig. 3C**). While 17-AAG only slightly decreased colony formation in SNCG transfected SNCG-435-3 cells, resulting in a 29% inhibition, treatment of parental MDA-MB-435 cells with 17-AAG resulted in a significant 74% reduction of colony formation. These data suggest that SNCG renders resistance to 17-AAG-induced apoptosis by protecting Hsp90 chaperoned client proteins.

Since SNCG renders cellular resistance to 17-AAG-induced apoptosis, we sought to determine whether this inhibitory effect of SNCG could be administered *in vivo* tumor xenograft model to a similar effect. It had been previously demonstrated that treatment of human breast cancer xenograft with 17-AAG (50 mg/kg, 3 times/week) can effectively suppress tumor growth at nontoxic doses and sensitize tumors to proapoptotic stimuli [34]. We treated mice with established MDA-MB-435 and SNCG-435-3 tumors with 17-AAG. As shown in **Fig. 4A**, treatment of MDA-MB-435 tumors resulted in 62% tumor growth inhibition. Consistent with cytotoxicity data in cell culture, SNCG-435-3 tumors were resistant to the treatment with only 25% tumor growth inhibition. A dose of 50 mg/kg 17-AAG was sufficient to cause a >90% reduction in HER2 expression and abolished activated Akt in MDA-MB-435 tumors. In contrast, expression of SNCG prevented HER2 degradation and restored Akt activation in SNCG-435-3 tumors (**Fig. 4B**). Since the 17-AAG treatment affects many of the Hsp90 client proteins, SNCG-mediated partial reversing the antitumor effect of 17-AAG might also be related to its chaperoning function on multiple Hsp90 client proteins.

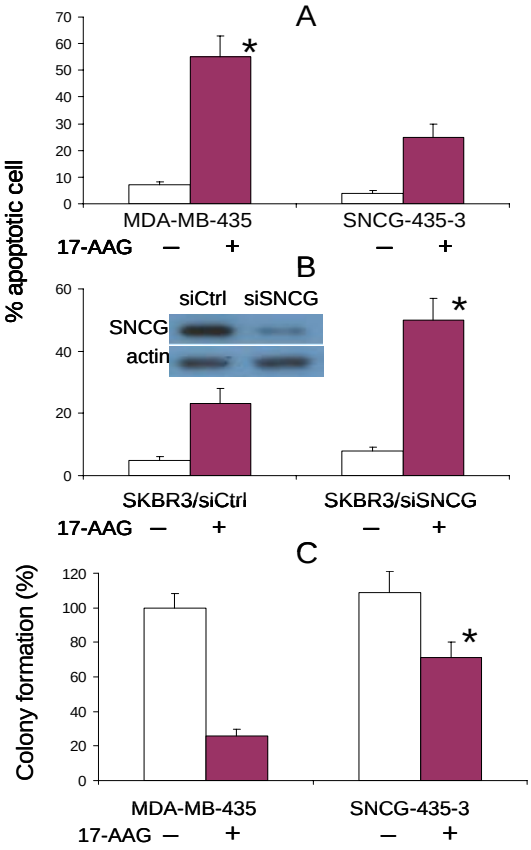


Fig. 3. SNCG renders cellular resistance to 17-AAG-induced cytotoxicity. **A**. MDA-MB-435 and its SNCG stably transfected SNCG-435-3 cell. **B**. Control SKBR3/siCtrl and its SNCG knockdown SKBR3/siSNCG cells. Insert is a Western blot showing knockdown endogenous SNCG expression in SKBR3/siSNCG cells, Cells were treated with 1 μM 17AAG for 4 days; change medium every two days. Apoptotic cells were determined 4 days following treatments. The numbers represent means ± SD of three cultures. *Statistical comparisons of apoptotic cells in SNCG-positive cells vs. SNCG-negative or knockdown cells indicate $p < 0.001$. **C**. Colony formation of MDA-MB-435 and SNCG-435-3 cells. Cells were treated with or without 1 μM 17AAG. Medium with fresh added 17-AAG was changed every three days. The number of colonies was counted after 2 weeks of plating. *Statistical comparison of colony formation for 17-AAG-treated MDA-MB-435 cells relative to 17-AAG-treated SNCG-435-3 cells indicates $p < 0.001$.

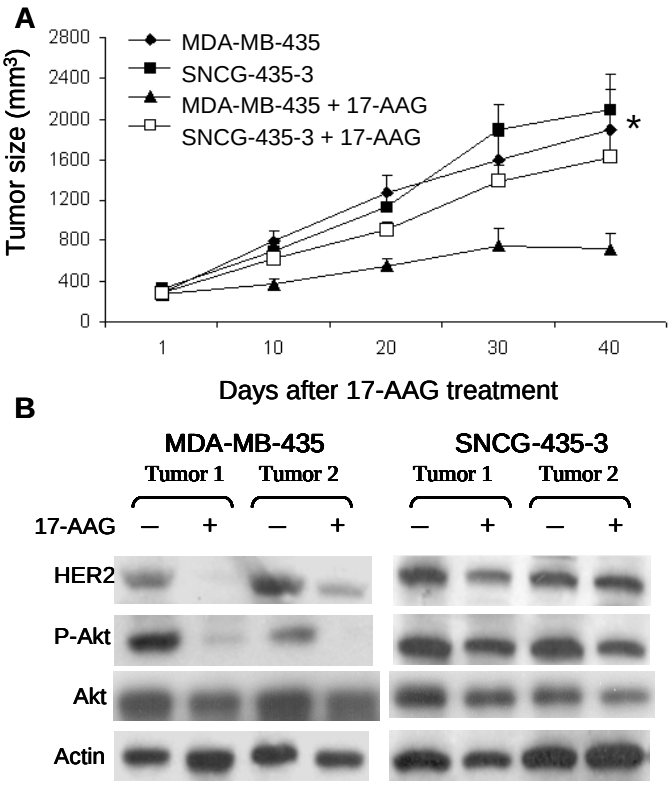


Fig. 4. **A**. Tumor growth in response to 17-AAG treatment. Nude mice were inoculated with approximately 1 x 10^6 MDA-MB-435 and SNCG-435-3 cells into the mammary fat pads. There were six mice in each group and each mouse received two injections, one on each side. Mice bearing established tumors were treated with either vehicle control or 17-AAG (50 mg/kg, i.p., 3 times/week). All mice were sacrificed at day 40 following the first drug treatment. Statistical comparison for tumor size in 17-AAG treated MDA-MD-435 mice relative to mice in other groups indicates *P <0.01. **B**. Protection of HER2 expression and activated Akt. Tumors were harvested from two MDA-MB-435 and two SNCG-435-3 xenografts at day 40, 12 hours following the last 17-AAG treatment. Total protein was subjected to Western analyses of HER2, phosphorylated Akt, Akt, and actin.

Expression of SNCG in mammary gland protects HER2 and exacerbates HER2-induced mammary tumor development. To study the *in vivo* biological relevance of SNCG on HER2 expression and function, we developed bitransgenic mice that coexpressed both HER2 and SNCG in the mammary epithelium by crossbreeding the previously established MMTV/SNCG transgenic mice [18] with MMTVneu HER2 transgenic mice. The effect of SNCG on HER2 was investigated in the mammary gland of SNCG/HER2 bitransgenic mice. To determine whether SNCG protects HER2 expression in the mammary gland, we first used *ex vivo* model involving a whole mouse mammary gland organ culture (MGOC) to study whether SNCG can prevent HER2 degradation due to the loss of Hsp90 chaperoning function. Treatment of mammary glands from HER2 transgenic mice in MGOC with 17-AAG caused a significant reduction of HER2 expression. However, consistent with the *in vitro* effect in breast cancer cells, co-expression of SNCG in HER2 transgenic mammary gland prevented 17-AAG-induced HER2 degradation, resulting in only a slight decrease in HER2 expression (**Fig. 5A**). We next studied the effect of SNCG on the expression and function of HER2 and Akt on the transgenic mice treated with 17-AAG. Treatment of HER2 transgenic mice with 17-AAG resulted in a significant loss of HER2 and inactivation of Akt. However, expression of SNCG in the SNCG/HER2 bitransgenic mice prevented the loss of HER2 and restored Akt activation due to the disruption of Hsp90 functions (**Fig. 5B**). These studies provided direct evidence supporting the chaperone role of SNCG on Hsp90 client proteins of HER2 and Akt in a more relevant mammary gland.

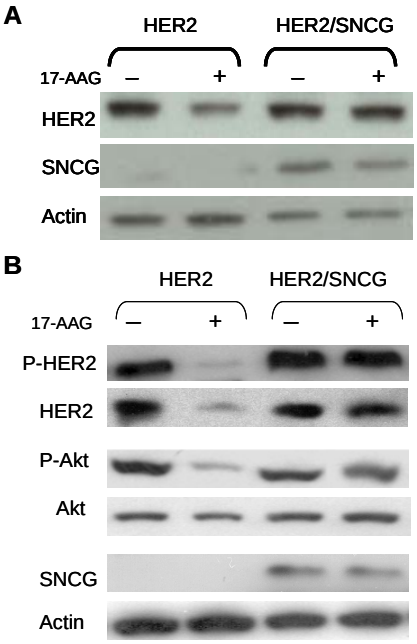


Fig. 5. **A**. Protection of 17-AAG-induced HER2 degradation by SNCG in mammary organ culture. A pair (left and right) of inguinal mammary glands from a 14-week virgin HER2 transgenic mouse and a HER2/SNCG bitransgenic mouse were cultured in the organ culture medium as described in Methods for 2 days followed by a 2-day treatment with 1 μM 17-AAG. The left mammary gland was used as control and the right gland was treated with 17-AAG. Total protein was isolated and subjected to Western analysis. **B**. SNCG protects HER2 and Akt in HER2/SNCG bitransgenic mice. A 14-month old HER2/SNCG bitransgenic mouse as well as an age-matched HER2 transgenic mouse were treated with 17-AAG (50 mg/kg, i.p., 3 times/week) for three weeks. Age-matched control mice were treated with vehicle (5% Tween 80, 5% DMSO in PBS). At the end of 3-week treatment, mice were sacrificed and the right inguinal gland was removed and subjected to Western analysis of HER2, Akt, SNCG, and actin. Activated HER2 and Akt were normalized with total HER2, Akt, and actin.

Effect of SNCG on HER2 induced mammary tumor was investigated in SNCG/HER2 bitransgenic mice. While expression of SNCG in mammary epithelium induces mammary hyperplasia, MMTV/SNCG transgenic mice fail to develop mammary tumors (15). Previous studies have shown that HER2 transgenic mice develop multifocal metastatic mammary tumors at ~6 months of age (16). Expression of SNCG in mammary glands significantly stimulated HER2-induced mammary tumorigenesis (Table 1). The results reveal that bitransgenics show significantly reduced latency of palpable mammary tumor formation with 50% of the animals showing tumor formation at 145 days as opposed to 182 days for HER2 mice. Tumor growth was also significantly stimulated. At day 250, the average tumor size of bitransgenics was 1.45-fold of that of HER2 mice. There was a slight increase in tumor incidence in bitransgenics, but it was not significant compared with HER2 mice. To determine whether 17-AAG has an antitumor effect on transgenic mice and whether expression of SNCG can render resistance to 17-AAG-mediated antitumor effect on HER2 mice, we treated the tumor-bearing mice with 17-AAG for 1-month period from day 220 to day 250. Tumors in HER2 mice receiving the

treatment had a mean 65% reduction in volume when compared with tumors in mice that were treated with vehicle control (318 mm^3 vs. 921 mm^3). In contrast, SNCG/HER2 mice treated with 17-AAG demonstrated a slight 22% tumor inhibition during the therapy (1045 mm^3 vs. 1338 mm^3). These data indicate that SNCG renders tumor resistance to Hsp90 disruption.

Experimental Group	Tumor Incidence	T50	Tumor Size (mm3)
Genotypes	Tumor/Total (%)	(Days)	Day 250
SNCG	N/A	N/A	N/A
HER2	52	182	921 ± 198
SNCG/HER2	58	145	1338 ± 281
With Treatment of 17-AAG at day 220-250			
HER2	N/A	N/A	318 ± 49 (65% ↓)
SNCG/HER2	N/A	N/A	1045 ± 136 (22% ↓)

Table 1. Expression of SNCG exacerbates HER2-induced mammary tumor development and renders resistance to antitumor effect of 17-AAG. The effect of SNCG on HER2-mediated tumorigenesis was measured by tumor incidence (percentage of mice developing tumors at 250-day period), tumor latency T50 (day with 50% of the animals showing tumor formation), and tumor size at the day 250. For treatment, mice bearing tumors were treated with either vehicle control or 17-AAG (50 mg/kg, i.p., 3 times/week) for 1-month period from day 220 to day 250. There were 15 mice analyzed for each group. All mice were sacrificed at day 250 following the first drug treatment. Tumor growth inhibition was calculated by comparison of treated vs. non-treated mice. Statistical comparisons for both T50 and tumor size in SNCG/HER2 bitransgenic mice relative to HER2 transgenic mice indicate $p < 0.01$. Statistical comparison for tumor incidence in SNCG/HER2 mice relative to HER2 mice indicates $p > 0.05$. Statistical comparison for tumor size in 17-AAG treated vs. non-treated HER2 mice indicates $P < 0.01$. Statistical comparison for tumor size in 17-AAG treated vs. non-treated SNCG/HER2 mice indicates $P > 0.05$.

KEY RESEARCH ACCOMPLISHMENTS AND REPORTABLE OUTCOMES. We provided evidences indicating that SNCG is a new member of molecular chaperone protein, which protects HER2 under stressful condition when the function of Hsp90 is disrupted.

1. SNCG prevents the degradation and the loss of activity of HER2 under stressful conditions when the chaperoning function of Hsp90 is blocked by 17-AAG. The SNCG-mediated chaperone activity was demonstrated in four different cell systems including overexpression of SNCG in SNCG-negative MCF-7 cells and MDA-MB-435 cells and knockdown endogenous SNCG expression in SNCG-positive T47D and SKBR3 cells.
2. Protection of HER2 and Akt by SNCG was also demonstrated in tumor xenograft and the mammary gland from HER2/SNCG bitransgenic mouse.
3. SNCG binds to HER2 oth *in vitro* in cell-free system and in breast cancer cells; such interaction occurs even in the presence of 17-AAG, in which Hsp90 dissociates from his client protein HER2.
4. Crossing SNCG transgenic mice with HER2 mice stimulated HER2-induced mammary tumor growth and rendered a resistance to 17-AAG-mediated antitumor effect.

5. Expression of SNCG renders resistance to 17-AAG-induced apoptosis both *in vitro* and in tumor xenograft. These data suggest a critical role of SNCG in maintaining the stability and function of HER2 ignaling pathway.

CONCLUSIONS

Synuclein gamma (SNCG), previously identified as a breast cancer specific gene, is highly expressed in malignant cells but not in normal epithelium. 17-AAG causes the degradation of HER2 and other Hsp90 targets and is the first Hsp90 inhibitor tested in clinical trials. The present study demonstrated SNCG can partially replace the chaperoning function of Hsp90 and protect HER2 stability and function in the stressful condition when the function of Hsp90 is blocked. Disruption of Hsp90 with 17-AAG resulted in a significant degradation of HER2. However, expression of SNCG completely recovered 17-AAG-mediated losses of HER2. This SNCG-mediated protection was demonstrated in breast cancer cells, tumor xenograft, and mammary glands from HER2/SNCG bitransgenic mouse. Consistent with its chaperoning activity, SNCG bound to HER2 n the presence and absence of Hsp90. While expression of SNCG renders resistance to 17-AAG-induced apoptosis both *in vitro* and in tumor xenograft, knockdown endogenous SNCG enhances the sensitivity to the Hsp90 disruption. Crossing SNCG transgenic mice with HER2 mice stimulated HER2-induced tumor growth and rendered resistance to 17-AAG-mediated antitumor effect on the transgenic mice. The present study indicates that SNCG functions as a tumor specific chaperone, which can replace the chaperoning function of Hsp90, protect its client protein HER2, and render a resistance to Hsp90 disruption.

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