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Synuclein gamma protects HER2 and renders resistance to Hsp90 disruption☆

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ABSTRACT

Hsp90 is an important driver of stabilization and activation of several oncogenic proteins in many key pathways in oncogenesis, including HER2. The present study demonstrated that synuclein gamma (SNCG) prevents the protein degradation and protects the function of HER2 in the condition when the function of Hsp90 is blocked. Disruption of Hsp90 resulted in a significant degradation of HER2 and the loss of activity. However, SNCG completely recovered Hsp90 disruption-mediated losses of HER2 and the function. SNCG bound to HER2 in the presence and absence of Hsp90. Specifically, the C-terminal (Gln106-Asp127) of SNCG bound to the loop connecting αC helix and β4 sheet of the kinase domain of HER2. SNCG renders resistance to 17-AAG-induced tumor suppression in tumor xenograft. Crossing SNCG transgenic mice with HER2 mice stimulated HER2-induced tumor growth and rendered resistance to Hsp90 disruption. The present study indicates that SNCG protects Hsp90 client protein of HER2, and renders resistance to Hsp90 disruption.

1. Introduction

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 (Neckers, 2007). Hsp90 keeps unstable proteins poised for activation until they are stabilized by conformational changes associated with signal transduction (Wandinger et al., 2008). So far, more than 200 client proteins are known to be regulated by Hsp90, including key mediators of signal transduction and aberrant oncogenic fusion proteins (Neckers, 2007; Wandinger et al., 2008; Bonvini et al., 2002; Nimmanapalli et al., 2001; Chiosis et al., 2001; Caldas-Lopes et al., 2009). Loss of Hsp90 chaperone activity may result in misfolding of target proteins, ultimately leading to their ubiquitylation

Abbreviations: BCSG1, breast cancer specific gene 1; ERα, estrogen receptor α; Hsp, heat shock protein; mTOR, mammalian target of rapamycin; SNCA, synuclein α; SNCB, synuclein β; SNCG, synuclein γ.

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and proteasomal degradation (Wandinger et al., 2008). Because of these activities, Hsp90 is becoming an emerging therapeutic target for cancer. The clinical development of Hsp90 inhibitors, such as 17-AAG, as cancer chemotherapeutics is progressing rapidly. Although 17-AAG, which permits simultaneous inhibition of multiple Hsp90 regulated client oncoproteins, is a promising new anticancer agent, acquired resistance to 17-AAG has been demonstrated in many different cancer cells (Workman et al., 2007; Gaspar et al., 2009; McCollum et al., 2006), indicating that other proteins or chaperones may also protect Hsp90 client proteins when the Hsp90 function is blocked.

We previously identified a breast cancer specific gene BCSG1, also known as synuclein γ (SNCG) (Ji et al., 1997). Synucleins are a family of small proteins consisting of 3 known members, synuclein α (SNCA), synuclein β (SNCB), and SNCG (Clayton and George, 1998). While synucleins are highly expressed in neuronal cells and have been specifically implicated in neurodegenerative diseases (Polymeropoulos et al., 1997; Spillantini et al., 1997), SNCG is not clearly involved in neurodegenerative diseases but is primarily involved in neoplastic diseases. So far, the abnormal expression of SNCG protein has been demonstrated in many different malignant diseases, including breast (Ji et al., 1997; Bruening et al., 2000; Wu et al., 2003; Guo et al., 2007), liver (Liu et al., 2005; Zhao et al., 2006), esophagus (Liu et al., 2005), colon (Liu et al., 2005, 2008, 2010; Hu et al., 2009), gastric (Liu et al., 2005), lung (Liu et al., 2005), prostate (Liu et al., 2005), pancreas (Li et al., 2004; Hibi et al., 2009), bladder (Iwaki et al., 2004), cervical cancers (Liu et al., 2005), ovarian cancer (Bruening et al., 2000; Czekierdowski et al., 2006), and glial tumors (Fung et al., 2003). In these studies, SNCG protein is abnormally expressed in a high percentage of tumor tissues but rarely expressed in tumor-matched nonneoplastic adjacent tissues. The clinical relevance of SNCG expression on breast cancer prognosis was confirmed in clinical follow-up studies (Wu et al., 2003; Guo et al., 2007). Patients with an SNCG-positive tumor had a significantly shorter disease-free survival and overall survival compared with the patients with no SNCG expression. SNCG is a new unfavorable prognostic marker for breast cancer progression and a potential target for breast cancer treatment. At the cellular level, SNCG increases metastasis (Jia et al., 1999) and hormone-dependent tumor growth (Jiang et al., 2003, 2004), promotes genetic instability (Gupta et al., 2003; Inaba et al., 2005), and regulates estrogen receptor signaling (Jiang et al., 2004; Liu et al., 2007; Shi et al., 2010).

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 (Jones and Buzdar, 2009). Considering that HER2 is an Hsp90 client protein and requires interaction with Hsp90 and its chaperone to acquire proper protein function (Citri et al., 2004), the Hsp90 inhibitor such as 17-AAG provides an alternative approach to target HER2 through dissociation of HER2 from the chaperone, which leads to HER2 degradation by a proteasome-dependent manner (Xu et al., 2001). Our previous studies demonstrate that expression of SNCG was strongly correlated with lymph node involvement, metastasis, and HER2 status (Guo et al., 2007). In the present study, we evaluated the effect of SNCG on HER2 expression and function. The results indicate that SNCG specifically interacts with HER2 and protects HER2 stability and function under stressful conditions when the activity of Hsp90 is blocked.

2. Results

2.1. *SNCG prevents Hsp90 disruption induced HER2 degradation*

Clinical studies demonstrated that expression of SNCG is clinically associated with HER2 status (Guo et al., 2007). Since the stability and function of HER2 is regulated by Hsp90 and 17-AAG acts primarily by promoting the degradation of HER2, we investigated whether expression of SNCG could prevent 17-AAG-induced HER2 degradation. Using previously established SNCG transfected MCF-7 cell (Jiang et al., 2003, 2004), 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 (Figure 1A). Treatment of the cells with Herceptin had no effect on HER2 expression levels. To investigate whether the reduction of HER2 was due to proteasomal degradation, we treated MCF-7 cells with proteasome inhibitor MG132. The results of this treatment revealed that 17-AAG-induced HER2 degradation could be blocked by proteasome inhibitor (Figure 1B). These results indicate that dysfunction of Hsp90 by treatment with Hsp90 inhibitors destabilized the HER2 protein and removed the unstable HER2 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 (Jia et al., 1999). As demonstrated in Figure 1C, knockdown SNCG in T47D cells significantly increased sensitivity to 17-AAG-induced HER2 degradation. Since SNCG activates ERα transcriptional activity (Jiang et al., 2003, 2004) 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 (Figure 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 down-regulation 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 (Chambers, 2009), next