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Induction of heat shock protein HSPA6 (HSP70B′) upon HSP90 inhibition in cancer cell lines

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ABSTRACT

Genome-wide transcript profiling to elucidate responses to HSP90 inhibition revealed strong induction of HSPA6 in MCF-7 cells treated with 17-AAG. Time- and dose dependent induction of HSPA6 (confirmed by qPCR and Western Blots) occurred also upon treatment with Radicicol, another HSP90 inhibitor. HSPA6 was not detectable in untreated cells or cells treated with toxins that do not inhibit HSP90, or upon applying oxidative stress. Thus, HSPA6 induction is not a general response to cytotoxic insults. Modulation of HSPA6 levels by siRNA-mediated inhibition or recombinant expression did not influence 17-AAG mediated cell death. HSPA6 induction as a consequence of HSP90 inhibition occurs in various (but not all) cell lines and may be a more specific marker for HSP90 inhibition than induction of other HSP70 proteins.

1. Introduction

Geldanamycin is a small molecule that inhibits the function of HSP90 family proteins [1,2]. As HSP90 is overexpressed in many tumors, Geldanamycin is a candidate for anti-cancer therapies [3], and efficacy of Geldanamycin derivatives is evaluated in numerous clinical trials [4–6]. Preliminary results suggest that growth of HER2-positive breast cancers is particularly sensitive towards HSP90 inhibition by the Geldanamycin derivative 17-AAG (Tanespimycin) [7]. This observation might be explained by the dependency on HER2 expression of this cancer subset as HER2 is a "client" protein of HSP90 [8]. HSP90 is a master regulator of HER2 protein stability, and inhibition of HSP90 function results in degradation of HER2 [8]. It appears, however, that some members of the Heat shock 70 protein family become induced in Geldanamycin treated cells which interferes with the therapeutic potency of Geldanamycin [9]. In complete agreement with that, a recent report demonstrates enhanced anti-cancer efficacy upon dual targeting of HSP90 and HSP70 in some cancer subtypes [10]. Therefore, a more detailed understanding of the interplay between members of the cellular HSP network in the context of HSP90-inhibition may improve anti-cancer therapies.

The HSP70 family comprises at least 13 members [11], of which HSPA6 (HSP70B') appears evolutionary unique as it is not conserved in rodents [12,13]. HSPA6 expression is not detectable in most cells under normal conditions. It becomes induced upon severe stress conditions and might mediate cytoprotective functions in a cell-type and context-dependent manner [11,14,15]. The potential influence of drug-mediated HSP90 inhibition on expression of HSPA6 has not been analyzed so far. This work describes the specific induction of HSPA6 upon treatment of cancer cells with HSP90 inhibitors.

2. Results

2.1. Geldanamycin induces the expression of HSPA6 in MCF-7 breast cancer cells

A genome-wide analysis of transcriptional responses in MCF-7 breast cancer cells was performed upon exposure to respective

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IC50 concentrations of the HSP90 inhibitor 17-AAG, in comparison to other cytotoxic compounds with cellular targets other than HSP90: these included alpha-amanitin (ama, targets RNA-Pol), saporin or cycloheximide (sap, CHX, both target the ribosome). We were able to recapture previously described Geldanamycin-mediated effects, such as the induction of various HSP70 and HSP40 family members [16] (Table 1), thus validating our experimental work-flow. Surprisingly, we also observed that HSPA6, a poorly characterized member of the HSP70 family became induced to higher levels (more than 3000-fold) than any other gene (up to 50-fold), even though induction of HSPA6 expression upon Geldanamycin treatment has not been described before. HSPA7 is very closely related to HSPA6 (the putative HSPA7 protein is 94% identical to HSPA6), but has been characterized as a pseudogene [13,17]. Therefore, the mRNA hybridization signals could have been derived from HSPA6 and/or HSPA7. To differentiate between these two transcripts, we performed qPCR with three different primer sets at different time points following 17-AAG treatment. Each of these primer sets amplified regions of HSPA6/HSPA7 with at least one nucleotide mismatch between HSPA6 and HSPA7 sequences. We were subsequently able to distinguish between HSPA6/HSPA7 transcripts by sequencing transcript fragments derived from qPCRs at 4 h time point (=maximal induction). This revealed that all amplified fragments exclusively contained HSPA6 sequences, indicating exclusive (or at least predominant) induction of HSPA6 and not HSPA7 (Fig. 1). These qPCR analyses also revealed that induction of HSPA6 transcripts is time-dependent. It peaks around 4 h and declines to low levels between 4 and 8 h after 17-AAG treatment (Fig. 1). Further, HSPA6 mRNA induction is dose-dependent and saturation is observed with concentration of 17-AAG between 125 and 250 nM (Fig. 1C). Next, we performed Western Blot analyses to test if GA-induced HSPA6 transcription translates to HSPA6 protein production. We separated 17-AAG treated and untreated MCF7 cell extracts on SDS/PAGE followed by immunoblotting with a HSPA6-specific antibody. HSPA6 protein is not detectable in untreated, but in 17-AAG-treated cells (Fig. 2A). Similar to our observations for HSPA6 mRNA levels, HSPA6 protein levels reach a plateau at around 4 h following 17-AAG treatment (Fig. 2A) and induction is concentration-dependent up to 500 nM 17-AAG (Fig. 2B). In contrast to other HSP70 proteins (the HSP70-antibody used in this study detects HSP72, HSPA1L as well as HSPA8), which remain significantly enriched even 24 h after 17-AAG treatment, HSPA6 protein levels decline rapidly between the 8 h and 24 h time points (Fig. 2A).

Taken together, we conclude that exposure of MCF-7 cells to 17-AAG leads to a transient induction of HSPA6 protein expression.

2.2. HSPA6 expression is specifically induced by HSP90 inhibitors

A comparison of the genome wide mRNA profiles of MCF7 cells treated with various toxins reveals that HSPA6 signals become induced only by 17-AAG exposure, but not by toxins with targets other than HSP90 (Table 1). This indicates that HSPA6 induction is not a general response towards cytotoxic insults, but instead either a specific consequence of treating cells with the substance 17-AAG, or triggered by inhibition of its molecular target HSP90.

In order to determine if induction of HSPA6 is a direct consequence of HSP90-inhibition, we examined the effects of Radicicol, another HSP90-inhibitor, on HSPA6 expression in MCF-7 cells. Similar to 17-AAG, Radicicol induced HSPA6 mRNA (Fig. 3) in a concentration-dependent manner. Maximum levels of induced HSPA6 transcripts appear somewhat lower in Radicicol- compared to 17-AAG-treated cells. This may be caused by the previously

Table 1
Induction of HSPA6 in 17-AAG treated MCF-7 cells. Cells were exposed for the indicated time periods to IC50 concentrations of toxins that either inhibit protein synthesis (Sap = Saporin, CHX = Cycloheximide), protein (re-)folding (17-AAG = 17-*N*-allylamino-17-demethoxygeldanamycin), or transcription (Ama = alpha-amanitin). Listed are log change values, i.e. fold changes are calculated according to the formula (Euler Constant)^{value}. Changes were observed with at least 2 probes for HSPA6/HSPA7, DNAJ and HSP90. HSP70A, HSP70B, and HSP70L were detected with one specific probe for each mRNA and one probe that detects HSP70A as well as HSP70B. Increased expression is indicated green (positive values), decreased expression in red (negative values), minor or no changes in yellow.

	17-AAG 2h	17-AAG 4h	17-AAG 7h	Ama 2h	Ama 4h	Ama 7h	Sap 2h	Sap 4h	Sap 7h	CHX 2h	CHX 4h	CHX 7h
HSPA6/HSPA7 (HSP70B')	**7.03** **6.53**	**8.30** **7.90**	**7.01** **6.50**	-0.03 -0.10	-0.03 -0.01	0.06 0.00	-0.02 -0.04	0.17 -0.03	0.31 0.09	-0.15 -0.12	-0.13 -0.18	-0.09 -0.10
DNAJB1 (HSP40)	**2.23** **2.16**	**3.00** **2.62**	**2.51** **2.33**	0.07 -0.21	0.19 -0.13	-0.12 -0.83	0.12 -0.11	0.52 0.31	0.57 0.27	-0.84 -0.92	**-1.36** **-1.36**	**-1.48** **-1.51**
DNAJA4 (HSP40)	**1.50** **1.21**	**3.01** **2.43**	**3.92** **2.98**	-0.52 -0.25	-0.41 -0.31	-0.48 -0.24	-0.17 -0.15	-0.14 -0.01	0.62 0.24	-0.21 -0.31	-0.77 -0.57	-0.78 -0.84
DNAJB4 (HSP40)	**2.17** **1.84**	**3.24** **2.88**	**3.06** **2.69**	-0.59 -0.57	0.08 0.23	0.31 -0.10	-0.40 -0.27	0.04 0.12	0.94 0.54	-0.44 -0.40	0.12 -0.06	0.41 0.17
HSPA1L (HSP70L)	**2,07**	**2,81**	**2,14**	-0,32	-0,03	0,06	-0,03	0,25	0,22	-0,77	**-1,12**	**-1,39**
HSPA1A/A1B (HSP70A+B)	**1,65**	**1,89**	**1,95**	-0,11	-0,14	0,25	-0,03	0,17	0,30	**-1,85**	**-2,77**	**-2,77**
HSPA1B (HSP70-B)	**1,29**	**1,54**	**1,77**	-0,19	0,10	0,19	-0,09	0,073	0,33	**-1,33**	**-2,10**	**-2,29**
HSPA1A (HSP70A/HSP72)	0,86	**1,04**	**1,21**	-0,10	0,02	0,06	-0,03	0,06	0,28	**-1,31**	**-2,23**	**-2,41**
Hsp90AA1 (HSP90A)	0,12 0.11	0,24 0.19	0,40 0.32	-0,07 -0.04	0,05 0.01	-0,06 -0.02	-0,04 -0.04	-0,02 -0.01	0,10 0.08	-0,06 -0.01	-0,07 -0.08	-0,11 -0.05
HSP90B1 (Grp94)	0,05 0.25	0,18 0.32	0,37 0.69	-0,09 -0.09	-0,04 -0.18	0,09 0.27	-0,01 -0.03	-0,04 -0.05	-0,06 -0.04	-0,06 -0.22	-0,16 -0.43	-0,18 -0.33

> +1.0	0.5 - 1.0	-0.5 – 0.5	-0.5 – -1.0	< -1.0