The impact of HSF1 or HSF2 knockdown on HSP90 inhibitor sensitivity in mammalian cells

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Introduction

Cancer cells are constantly in a stressed state and therefore, they are dependent on stress-protective proteins, such as heat shock protein 90 (HSP90). Thus, many attempts to develop a HSP90 inhibitor have been made. Current HSP90 inhibitors are problematic as they trigger the heat shock response (HSR) increasing the overall amounts of stress-protective proteins, which is counterproductive in cancer treatment (Kijima *et al.* 2018). The HSR is regulated by heat shock factor 1 (HSF1) and modulated by HSF2 that both have been linked to cancer (Gomez-Pastor *et al.* 2018). The goal of the project is to study if HSF1 or HSF2 knockdown is related to the decreased viability as a result of HSP90 inhibitor treatment. Since the biggest issue with existing HSP90 inhibitors is the counterproductive HSR induction, it is important to better understand the link between HSF1, HSF2 and HSP90 inhibition.

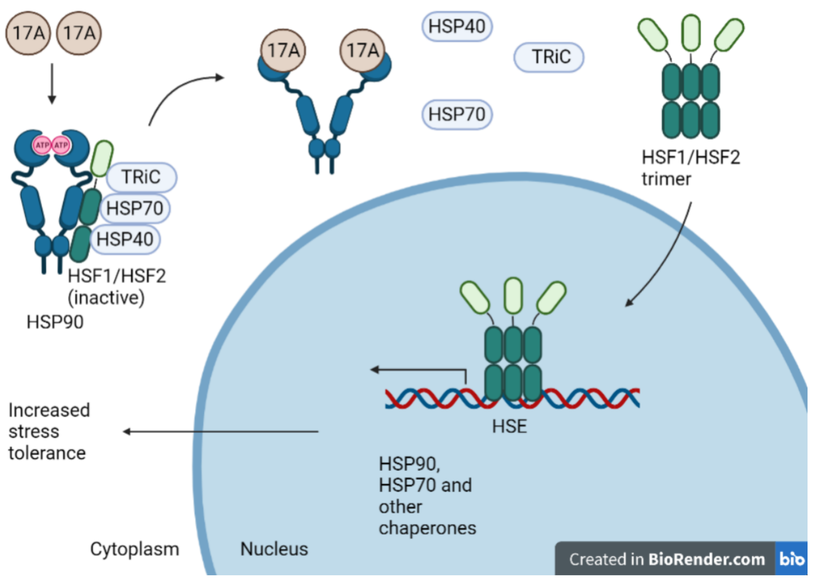

Figure 1. Binding of HSP90 inhibitor 17-AAG (17A) to HSP90 leads to dissociation of HSF1 or HSF2 from HSP90 complex. HSFs form a trimer and localize to the nucleus where they bind heat shock elements (HSE) and induce the expression of HSP90, HSP70 and other chaperones. Modified from Gomez-Pastor *et al.* 2018.

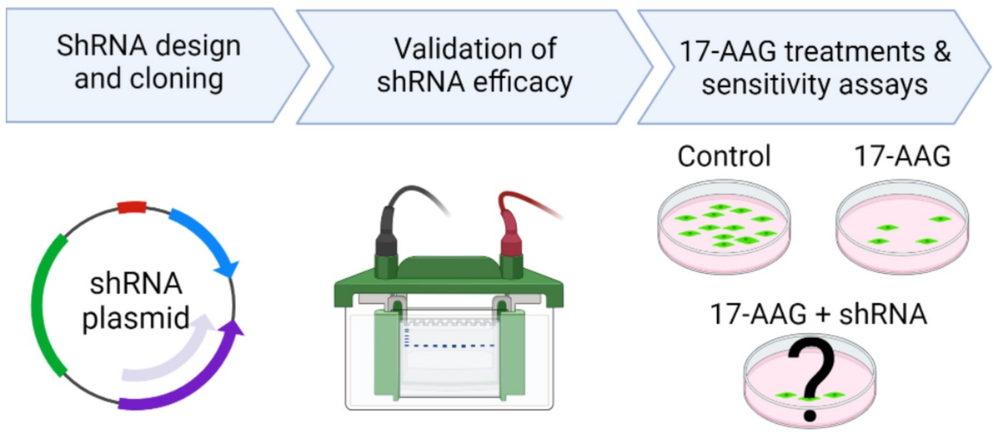

Figure 2. Workflow of the project. ShRNA plasmids against HSF1 and HSF2 were designed and cloned. Next, they were validated by Western blotting. The aim is to examine the cell viability of shRNA transfected cells after a two-day 17-AAG treatment in sensitivity assays.

Materials and Methods

The impact of HSF1 and HSF2 on cell viability upon HSP90 inhibitor treatment will be evaluated by reducing protein expression levels of HSF1 and HSF2 using short hairpin RNA (shRNA), a type of RNA interference. Plasmids that express shRNA and green fluorescent protein (GFP) as a marker were transfected into mammalian cells using electroporation (Neon transfection system by Thermo Fischer).

Results

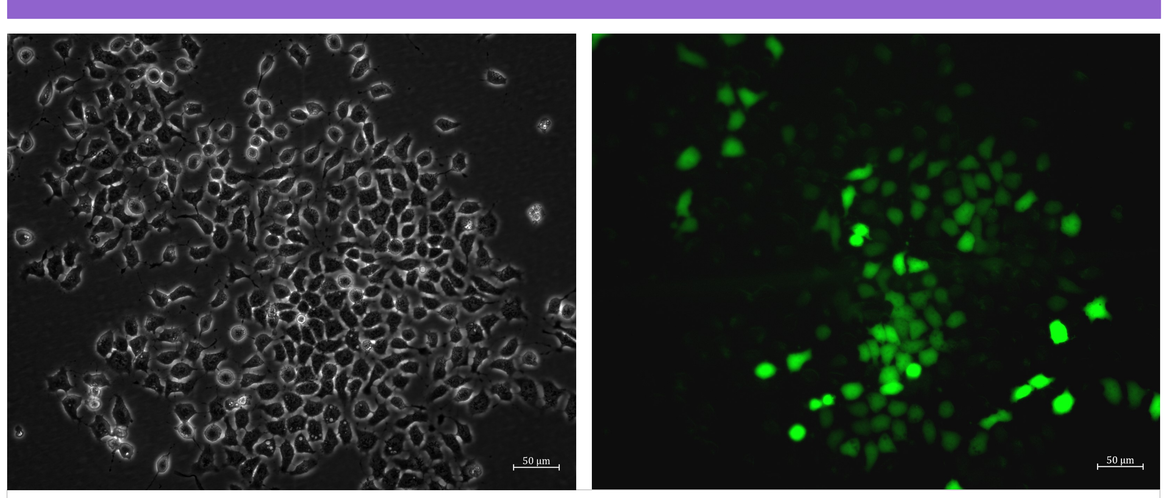

Figure 3. Transfection efficiency was validated in F9 cells by utilizing the green fluorescent protein (GFP) marker expression from the shRNA plasmid. The brightfield picture (left) was compared to the GFP picture (right) and based on that, the average transfection efficiency was ~50 %.

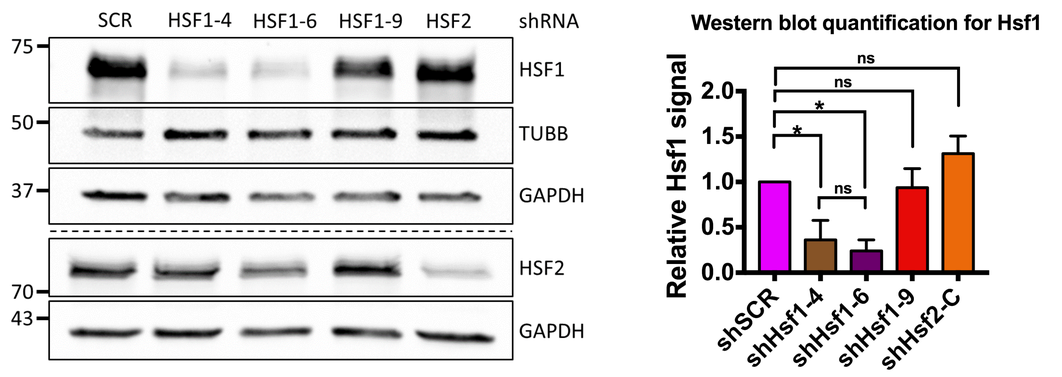

Figure 4. ShRNAs 1-4, 1-6 and 1-9 against HSF1 were first designed and then validated using F9 cells in Western blotting. Tubulin (TUBB) and GAPDH were used as loading controls. According to quantification, shRNAs 1-4 and 1-6 reduced the levels of HSF1 significantly and are equally effective. On the contrary, HSF1 levels were not affected by shRNA 1-9. Additionally, shRNA against HSF2 was used as a positive control for HSF2 downregulation. None of the HSF1 shRNA affected the levels of HSF2. * = $p < 0.05$, ns = not significant.

Upcoming experiments

The impact of a two-day treatment with HSP90 inhibitor 17-AAG on cell survival will be compared between several cell lines from human and mouse. Finally, the 17-AAG treatment will be combined with HSF1 or HSF2 downregulation to evaluate if cell viability is affected. The study will help to uncover why HSP90 inhibitors cause HSR and based on that, better HSP90 inhibitors can be developed.

References:

Gomez-Pastor, R., Burchfiel, E. T., & Thiele, D. J. (2018). Regulation of heat shock transcription factors and their roles in physiology and disease. *Nature reviews Molecular cell biology*, *19*(1), 4-19.

Kijima, T., Prince, T. L., Tigue, M. L., Yim, K. H., Schwartz, H., Beebe, K., ... & Neckers, L. (2018). HSP90 inhibitors disrupt a transient HSP90-HSF1 interaction and identify a noncanonical model of HSP90-mediated HSF1 regulation. *Scientific reports*, *8*(1), 1-13.

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