Detection and Relative Quantitation of Changes in Gene Expression of Heat Shock Proteins 27 (HSP27), HSP 70, HSP 90 and HSP 47 in Doxorubicin-Exposed Human Cells by RT-qPCR

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Introduction - Heat shock proteins (HSPs)

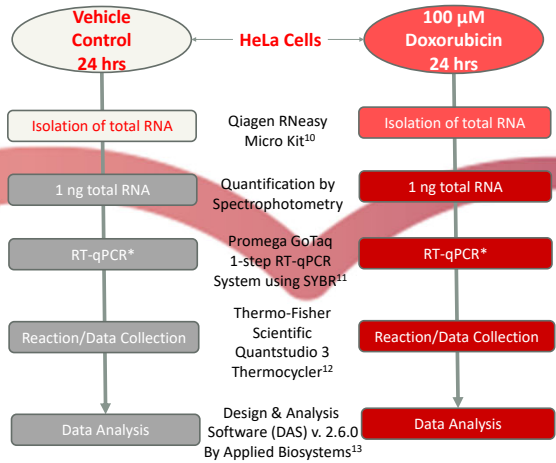
- Initially identified to be over-expressed during elevated temperature, and subsequently also during various stressful conditions including exposure to hypoxia and chemotherapeutic agents.[1]
- Molecular chaperones[1,2]
- Members of the HSP family are classified based on their molecular weight (in kDa)[3]
- **HSP70** and **HSP90** function in the regular folding and maturation of newly synthesized cellular proteins, and are associated with poor prognosis in breast cancer metastasis[4,5]
- **HSP27 (heat shock protein beta-1, HSPB1)**
  - Stabilizes denatured proteins and return them to the original form
  - HIGHLY expressed in aggressive cancers[6-8]
    - Alters the tumorigenesis and metastasis of tumor cells, including breast cancer cells
    - Confers chemoresistance
- **HSP47 (SERPINH1)**
  - Cellular protein involved in collagen synthesis
  - Confers chemoresistance on pancreatic cancer cells[9]

Purpose and Hypothesis

- **To detect and quantify the relative changes in gene expression of HSP90, HSP70, HSP27 and HSP47 with the chemotherapeutic drug doxorubicin using an *in vitro* cancer cell culture**
- **The HSP mRNA are hypothesized to be increased in the drug-treated samples**

Method

- *In vitro* Hela cells in cell culture were selected as a model and were maintained under standard conditions
- Procedures



Target Genes	Forward PCR Primers	Reverse PCR Primers	Ref.
GAPDH**	AATGCATCCTGCACCACCAA	GTAGCCATATTCATTGTCATA	14
Beta-Actin**	CCCAGCCATGTACGTTGCTA	TCACCGGAGTCCATCACGAT	15
HSP90	CGCTCCTGTCTTCTGGCTTC	TGGTATCATCAGCAGTAGGGTCA	15
HSP70	CCATCATCAGCGGACTGTACC	CTGACCCAGACCCTCCCTT	15
HSP27	AGGATGGCGTGGTGGAGA	GGGAGGAGGAAACTTGGGTG	14
HSP47	ATGCAGAAGAAGGCTGTTGC	GGCCTTGTTCTTGTCAATGG	16

***RT-qPCR reaction conditions: 37°C for 20 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by a melt curve analysis using a total RT-qPCR reaction volume of 20 µL[11,12]**
**** Internal control**

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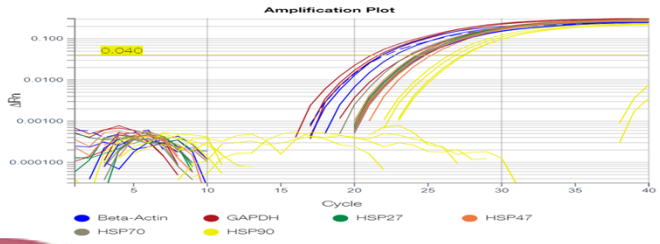
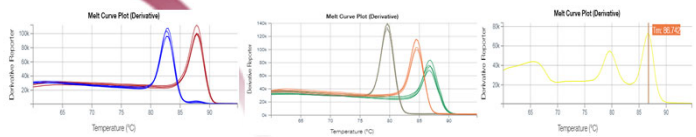
Results

- **Quality of RNA: A260/A280 ratio >1.8 in all samples**



Melt-Curve Analysis for SYBR reactions



GAPDH and Beta-Actin | HSP27, 47 and 90 | HSP70

Relative mRNA Quantitation between doxorubicin- and vehicle-treated cells (using beta-actin as internal control)

GAPDH	HSP27	HSP90	HSP47
96%	42.6%	46.6%	53%

Discussion and Conclusion

- mRNA of all target genes, except HSP70, were reliably detected using the current methodologies
- HSP70 reaction requires further primer/reaction optimization
- The decreases in HSP mRNA in the doxorubicin-treated HeLa cells were unexpected, appear to decrease by about half across the HSPs
  - This observation may be attributed to the duration of drug exposure

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