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SJSU Undergraduate Research Grants

Comparative Expression of SIRT4 in Cancerous vs. Non-Cancerous Human Mammary Cells

Arshia Hamzehpour S.[1,2], Aeowynn J. Coakley[1], Kenneth Lum[1,2], and Frank K. Huynh[1]

[1]Department of Biological Sciences, San Jose State University

[2]SJSU Undergraduate Research Grant Recipient

INTRODUCTION

Sirtuin 4 (SIRT4) is a deacylase enzyme that removes post-translational modifications from proteins in mitochondria and has a critical role in cellular metabolic regulation. Prior studies have shown that SIRT4 may play a role in certain cancers, such as breast cancer. Paradoxically, some studies have demonstrated that SIRT4 may have a tumor promoting effect in breast tissue, while others show SIRT4 as having a tumor suppressing effect. Interestingly, our preliminary data show that SIRT4 has a role in promoting normal mammary development. As normal mammary development and breast cancer development have overlapping pathways, we seek to further clarify the role of SIRT4 in these pathways.

RESEARCH QUESTIONS

We hypothesize that SIRT4 plays a critical role in regulating the metabolism of normal versus cancerous growth in mammary glands.

We are investigating SIRT4 expression in human mammary cancerous (MCF-7) and non-cancerous (HMEC) cell lines with the intention of quantifying gene and protein expression levels.

METHODS

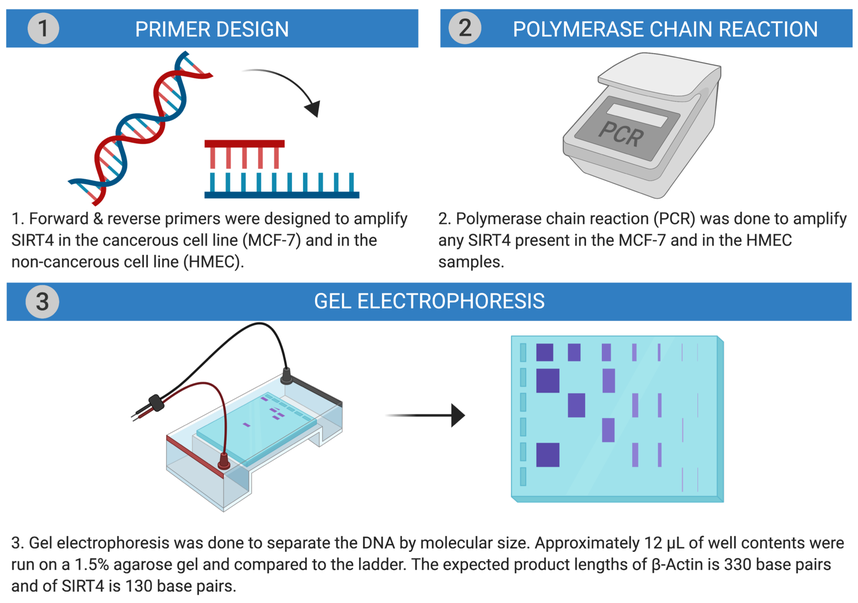

Figure 1. Experimental methods: 1) primer design 2) polymerase chain reaction 3) gel electrophoresis

PRIMER DESIGN

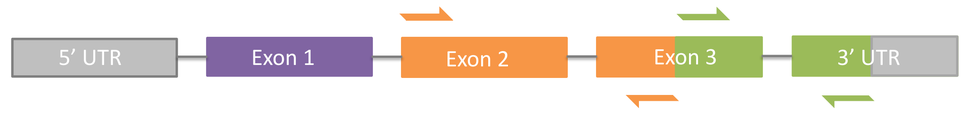

Figure 2. Location of both options of primer sets (forward and reverse) on the SIRT4 gene. Option 1) **hSIRT4.3 (forward) and hSIRT4.4 (reverse)** spanning exon 2 and exon 3. Option 2) **hSIRT4.5 (forward) and hSIRT4.6 (reverse)** and spanning exon 3 and the 3′ UTR region.

Table 1. Primer parameters. Parameters considered when designing primers. Ideal primer parameter is given, followed by characteristics of hSIRT4.3 & hSIRT4.4 and hSIRT4.5 & hSIRT4.6.

Primer Parameters							
Primer	**Sequence 5'-3'**	**Length (base pairs)**	**T_m (°C)**	**GC content (%)**	**Homodimer: minimum ΔG (kcal/mol)**	**Heterodimer: minimum ΔG (kcal/mol)**	**Product Length (base pairs)**
Ideal	---	~20	55-65	40- 60	> -9	> -9	100–200
hSIRT4.3	AAGAAGCCGACTCCCTCTTG	20	64.3	55.0	-5.12	-5.12	151
hSIRT4.4	TTCTTCTCCCAGGCAGTGAG	20	64	55.0	-3.17	-3.17	151
hSIRT4.5	AGAGTTGCTGCCTTTGATAGA	21	62.3	42.9	-3.14	-3.14	130
hSIRT4.6	GAATGGGAACTGGAATCTGTC	21	61.2	47.6	-1.57	-1.57	130

PRIMER OPTIMIZATION

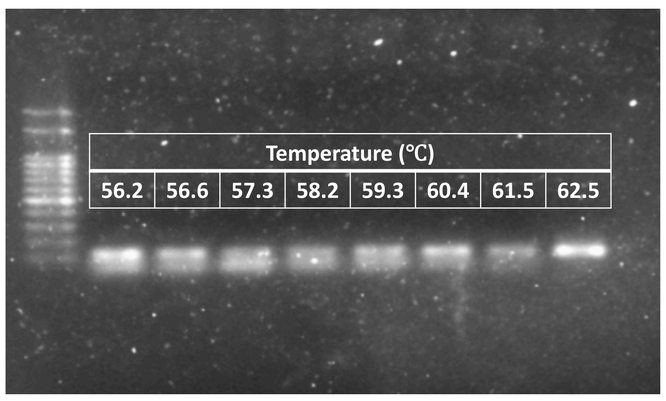

Figure 3. Primer temperature gradient. Temperature gradient PCR was performed to find the optimum temperature for the hSIRT4.5 and hSIRT4.6 primers in the MCF-7 cell line. DNA ladder for comparison purposes is shown in the well furthest to the left, and the temperature increases from left to right across the wells. 62.5 °C was selected as the optimum primer temperature because of how comparatively strong the expected product appeared and of the lack of primer dimers.

NEXT STEPS

Perform PCR to verify SIRT4 gene expression in MCF-7 cell line versus the HMEC cell line.

Perform quantitative PCR (qPCR) to quantify the amount of SIRT4 gene expression in MCF-7 cell line versus the HMEC cell line.

Perform Western blots to measure SIRT4 protein expression in MCF-7 cell line versus the HMEC cell line.