

# Effect of the Klotho Protein on the Expression of a Panel of Telomere Relative Genes

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## Introduction

- In mice, the over-expression of the Klotho gene extends the life span, whereas mutations to the Klotho gene shorten the life span. The human Klotho gene encodes the  $\alpha$ -Klotho protein.
- It is known that telomeres regulate the cellular aging process.
- This study was designed to interpret the function of the Klotho protein and how it may play a critical role in cellular life extension by regulating telomere length and activity. The theory is that other significant genes which are relative to telomere life may be affected and either an increased or decreased in the expression of other relevant genes.

## Process

- Construct a klotho containing plasmid vector with mammalian selection markers
- Transfect the primary adult mesenchymal stem cell lines from adipose tissue with the gene promoter to upregulate the Klotho gene.
- Generate a skin cell line that both expresses and secretes the klotho protein in the cell conditioned medium
- Detect and quantify the Klotho protein in the cell conditioned medium produced by the Klotho expressing cell lines. This cell conditioned medium is Advicell K

## Development of Klotho overexpressed mesenchymal stem cell in their cell conditioned medium (Advicell K)

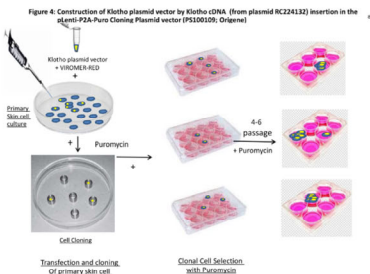
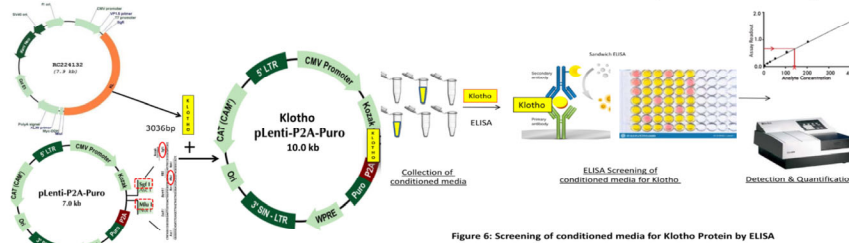


Figure 5: Klotho cell line generation: Transfection of Klotho plasmid DNA in primary cells & Klotho cell line selection

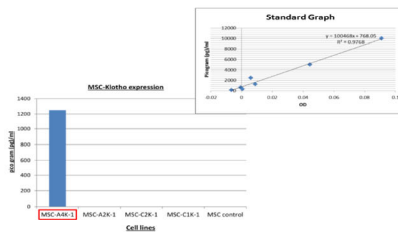


Figure 7: Conditioned media produced by MSC-A4K-1 cell line contain high level of Klotho protein

The objective of this study was to measure the effect of the test material listed in Table II on the expression of a panel of genes of interest, using quantitative PCR.

GENE	Advicell K 4%		Advicell K 0.8%		Advicell K 0.04%	
	p value	Fold change vs. water	p value	Fold change vs. water	p value	Fold change vs. water
MMP1	<b>0.000</b>	<b>10.2</b>	<b>0.010</b>	<b>1.9</b>	0.561	1.1
SIRT1	0.230	-1.2	0.336	1.2	0.783	-1.0
PTGS2	0.438	-1.2	0.813	1.1	0.901	-1.0
MMP9	0.017	-1.3	0.015	1.3	0.979	-1.0
SIRT6	0.388	-1.2	0.280	1.3	<b>0.057</b>	<b>1.7</b>
TLR6	<b>0.040</b>	<b>-2.2</b>	0.332	-1.3	0.638	-1.1
VEGFA	0.684	1.1	0.429	1.3	0.600	1.2
hTERT	<b>0.091</b>	<b>-1.5</b>	<b>0.194</b>	<b>1.5</b>	<b>0.170</b>	<b>1.5</b>

Table II. Effect of the different experimental conditions on the expression of a panel of genes of interest in aHDF cells. Modulatory conditions are labeled in bold characters.

## Materials & Methods

### Preliminary Experiment

- The effect of the test substance on cell viability being unknown, normal adult human dermal fibroblasts were incubated with the non-diluted (100%) Advicell K, as well as with its 20%, 4% and 0.8% aqueous dilutions.
- After an overnight incubation, cells were observed and the selection of doses for the principal experiment was made based on the assessment of cell morphology under different experimental conditions.

**The highest tested concentration** of Advicell K (4%) induced a strong expression of the matrix metalloproteinase MMP1. Although MMPs are required for recycling of old and damaged components of cells and the extracellular matrix (ECM), the upregulation of MMP1 is not generally viewed as beneficial in the cosmetic industry.

- Two other genes, coding for TLR6 and hTERT, have been downregulated by the highest tested dose of AdvicellK. TLR6 is a transmembrane receptor important for innate immunity. hTERT is a catalytic subunit of the enzyme telomerase, responsible for the maintenance of chromosome terminal sequences (telomeres). It protects telomeres from damage, fusion with neighboring chromosomes and from shortening leading to cellular senescence.

**The medium concentration** of Advicell K (0.8%) also increased MMP1 expression but to a much lesser extent than the highest dose (2 times vs. 10 times). hTERT was not repressed, but, to the contrary, the expression of this gene was moderately increased. Taken together, these data indicate a positive trend, making the analysis of the expression profile of the lowest tested dose particularly interesting

**The lowest tested concentration** of Advicell K (0.04%) showed the continuation of the trend observed with the two higher doses. The expression of MMP1 was totally normalized, while the heightened expression of the telomerase reverse transcriptase was maintained, with slightly improved value.

Furthermore, the dilution of AdvicellK to 0.04% unmasked the upregulation of another important gene – SIRT6. Sirtuin 6 is a stress-responsive protein implicated in telomere maintenance, caloric restriction, and life span expansion

**Conclusion** This project allowed to uncover valuable potential properties of AdvicellK towards telomere protection and cellular life extension – two activities at the forefront of cosmetic sciences.