Klotho containing serum induces a robust protective response in human skin cells measured using a novel screening platform.

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The skin is the largest organ in the body and the only one to come into contact with solar UV radiation (UVR). The UVA spectrum makes up over 95% of solar-UV radiation energy reaching the earth's surface. UVA (300-400 nm) has been recognized as a significant contributor to UV-related skin damage. UVA exposure can cause the induction of DNA damage including CPD and 8-oxo-G, as well as single-stranded breaks and apurinic (AP)-sites, increasing the incidence of skin cancer and accelerating photo-aging. The skin is unique in that it can succumb to the effects of chronological aging compounded by UVA induced photoaging. Sunscreens in the US must be broad spectrum, containing UVA and UVB blockers. Of the 12 chemical blockers available to the US sunscreen market only avobenzone is active in the UVA1 range (320- 400nm). Hence there is a dire need to find new compounds that reduce skin photoaging and are cosmetically elegant. Here we describe a novel klothos containing compound that not only reduces age related endogenous DNA damage in skin cells, but also significantly abrogated DNA damage levels induced by exogenous UVA1 (365nm) exposure. Overexpression of the Klothos gene was previously shown to extend lifespan in transgenic mice and give them a youthful appearance compared to litter mates. This was demonstrated to be in part due to the protein inducing protective cellular response pathways including potent antioxidants proteins superoxide dismutase and catalase.

Using a novel high-throughput UVR DNA damage assessment platform (UValidate, NIH supported) we characterized the impact of the proprietary klotho containing serum measuring aspects related to both chronological and photoaging. UValidate™, is comprised of three sub-systems automating the analysis and quantitation of nuclear DNA damage from UVA1, UVA2, UVB or a combined UVR solar simulation. The system is compatible with 2D and 3D cell and tissue cultures as well as patient derived skin samples. In this assessment 2D human derived fibroblast cells were pretreated with klotho serum and the levels of oxidative DNA damage measured under endogenous conditions. We measured the DNA damage levels in over 50K individual cells repeated in biological triplicate. We report that the klotho serum was able to reduce the levels of oxidative DNA damage significantly ($p > 0.001$) by approximately 40% compared to passaged matched isogenic controls. This suggested that the protective response induced by klotho is active and robust in these skin cells. Given this overwhelming positive response we went further and exposed the fibroblasts to UVA1 (365nm) at levels comparable to 8hrs exposure on a sunny day. These levels did not induce UVA related cell death but did induce a significant increase in UVA-related oxidative DNA damage. Cell treated with 1% klotho serum had a significant decrease in UVA related DNA damage levels. This reduction was not simply attributed to lower endogenous or starting levels of DNA damage but rather a clear protective response against the oxidative DNA damage induced by the UVA exposure. In previous research Klotho protein and second-generation growth factors in serum were found to be highly effective in improving the visible signs of photoaging include texture and wrinkles. In the present research we go further and look at the protective molecular response that klothos is able to induce in skin cells and report that klotho is an excellent candidate for both anti-aging and photodamage reduction interventions.