

DNA Human Gene Expression Summary for DC Upregulex

Spider silk has been known for its extraordinary properties for thousands of years. In fact, spiders have been using their own high-tech fibers to produce nano-based functional materials for centuries. These nano-protein-based-fibers have unique mechanical properties which have been designed through evolution to produce a protein strand stronger than collagen, even Kevlar. Recently, biological testing of these fibers on humans have shown that the fiber is immunologically compatible with human skin. This led to the recent practice of successfully growing keratinocytes (skin cells) in culture on silk fiber beds (in lieu of collagen). These fibers are currently being investigated in the field of pharmaceutical drug delivery, given the compatibility of these nano-protein fibers with human skin and other tissues.

DC Upregulex ingredient for skin care

Given these facts, we have tested hydrolyzed sericin (obtained from spent silk worm cocoons), solubilized in an efficient and unique delivery system (phospholipids multi-layer vesicle), for its affect on the gene expression of human skin. DNA micro array technology gives us the opportunity to visualize the changes in expression of all genes identified to date by measuring production of mRNA, the precursor molecule to proteins in the skin.

The activity of the extract, as measured by cDNA microarray analysis of the effects on mRNA production in full thickness epidermal tissue, exhibits distinct affects. Using a “norm” of 30% as a standard measure of significance (that is to say that a change in gene expression of 30% must occur to be a significant effect), 116 genes were upregulated and 28 genes downregulated by the addition of the extract. In particular, upregulation of genes involved in cell division, protection of dividing cells against apoptosis and dermal remodeling were observed.

Cellular Proliferation

The genes affected in the cell cycle by the addition of this extract are not “housekeepers”. They instead control many of the paramount regulatory functions involved in the cell division and proliferation. Primary affects were found to be at the G1 to S phase switch, driving the cell to DNA replication and committing the cell to division, proliferation and growth. M phase to G2 affects were also observed. System checks and balances that maintain DNA integrity during replication and cell division were maintained in the process. Predictably, this extract acts as a stimulus for the proliferation of non-quiescent cells. A list of those genes affected and a summary of their functions is listed at the end of this communication.

It is well known that with an increase in cellular proliferation, there is also an increase in the concentration of reactive oxygen species. DNA that is replicating or dividing is at its weakest state with regards to attack by ROS and must be protected to ensure integrity to the replication / division process. The proliferation-induced increase in ROS can cause DNA damage and induce pre-mature apoptosis or cellular / tissue death. Therefore, anti-apoptotic effects are of principal value in DNA protection against mutations during replication and cell division. This extract acted as a stimulus for cellular proliferation but also protected the tissue by inducing genes involved in the anti-apoptotic affect.

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The anti-apoptotic down-regulation of TRAIL, a potent pro-apoptotic stimulus, was observed. This effect occurs only in the presence of proliferating cells as this regulator deletes proliferating cells during cell division when left unchecked.

Although protection from a pre-apoptotic effect was observed for proliferating cells, it is also well known that quiescent cells can undergo damage during proliferation in the presence of cellular-proliferation-induced-ROS. This issue causes mutations to these cells and possible exit from quiescence. Such cells survive and exhibit uncontrolled and damaging growth patterns and cytokine signaling due to mutation. The tissue counteracts a possible pre-apoptotic affect to quiescent cells in growing tissues by upregulation of MgSOD. Increased resistance to oxidative stress is associated with longevity. It has been concluded that this model also extends to mammalian systems. It has recently been concluded that the induction of this enzyme decreases with aging.

Changes in gene expression due to the application of the extract also provided quiescent cells protection against apoptosis in a proliferating tissue. A significant increase in the induction of MgSOD was observed. Thus, the extract protects both proliferating and quiescent cells of a tissue during the activation of the cell cycle and mitosis.

Tissue Remodeling

PGE2 receptors are often thought to be involved in only the inflammatory response. But a second response to PGE2 during cellular proliferation is a non-inflammatory effect. In these cases, PGE2 effects induce expression of early growth response 1 (EGR1) and regulation of the level and stability of cyclooxygenase-2 mRNA, a potent regulator of collagen remodeling. An upregulation of the PGE2 receptor was observed, seemingly not involved in an inflammatory response as no inflammatory genes were induced. Thus, an increase in PGE2 efficacy due to an increase in receptor concentration was, in this case, a beneficial effect. This also leads to a premise that the affect of the extract may be primarily at the epidermal / dermal junction, as the genes involved affect primarily the extra cellular matrix.

The down-regulation of cellular communication was also observed. This must occur when cell division is pending as the individual cell separates itself from the tissue during division and proliferation.

Taken together, the proliferation of dermal cells (stimulated by cell cycle upregulation) would allow for the both healthy growth and migration of the fibroblasts in an antiapoptotic environment (stimulated by MgSOD upregulation and TRAIL downregulation). These are events that must occur during the process of wound healing. Therefore, this extract may be a valued tool for topical use on skin that has been wounded by trauma (cuts, scar forming environments, stress marks forming during pregnancy) as a dermal repair system. Other tests conducted independently form these experiments confirm an increase in glycosaminoglycan systhesis as well as collagen production upon long term application resulting in more elastic and resilient skin.

UPREGULATED GENES - CELL CYCLE

The most prevalent effect of the extract was on the cell cycle. In particular, the effect was observed to be on the ubiquitination mediated proteolysis pathway as well as important check

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points in the cell cycle. For the most part, genes involving the G1 to S phase switch in mitosis were affected.

A summary of the activity of each of these genes is as follows:

The origin recognition complex (ORC) – subunit 1 (inducible)

ORC is a highly conserved six subunits protein complex essential for the initiation of the DNA replication in eukaryotic cells. Studies demonstrate that ORC binds specifically to origins of replication and serves as a platform for the assembly of additional initiation factors such as Cdc6 (ORC activation) and then Mcm proteins to complete the DNA recognition complex essential for DNA replication during S phase. The protein encoded by this gene is the largest and most important subunit of the ORC complex. While other ORC subunits are stable throughout the cell cycle, the levels of this protein vary during the cell cycle. The concentration of this subunit in a cell has been shown to be controlled by ubiquitin-mediated proteolysis after initiation of DNA replication. This protein is found to be selectively inactivated by phosphorylation during M phase. It is also reported to interact with MYST histone acetyltransferase 2 (MyST2/HBO1), a protein involved in control of transcription silencing during replication to ensure integrity to the replication process.

CDC6 - Involved in the initiation of DNA replication, this protein also forms the DNA pre-recognition complex with ORC and activates S phase and DNA replication. Also participates in checkpoint controls that ensure DNA replication is completed before mitosis is initiated. Also forms the checkpoint to ensure that replication is complete and successful before ubiquitin mediated proteolysis and deactivation of S phase activators occurs.

POLO Kinase 1 – This protein is known to be required for cell division during G1 and S phase. The concentration of this protein begins to accumulate during S phase to a maximum during the G2 and M phases, declines to a nearly undetectable level following mitosis and throughout G1 phase. Induction of this gene occurs only in the presence of growth-stimulating agents.

S Phase Kinase Associated Protein 2 (p45) is the substrate recognition component of the SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins involved in cell cycle progression, signal transduction and transcription. In particular, this protein is upregulated in order to specifically recognize and initiate degradation of p27, a potent cell cycle inhibitor of the G1 to S phase switch. It allows recognition of the phosphorylated CDKN1B/p27kip complex and causes its removal from the cell to regulate the G1/S transition.

CDC20 acts as a regulatory protein interacting with several other proteins at multiple points in the cell cycle. Synthesis is initiated at G1/S, peaks in M phase and degrades abruptly at M/G1 transition. . It localizes in the cell nucleus during G1, but translocates to the cytoplasm at the start of S phase, a process regulated through its phosphorylation by Cdks, in particular maturation promoting factor (MPF).

In G1 to S phase transitions, it is involved in both the initiation of DNA replication as well as

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checkpoint controls that ensure DNA replication is completed before mitosis is initiated.

Also, this protein acts primarily to activate proteolysis of M phase cyclins and trigger anaphase in a dividing cell. Required for full ubiquitin ligase activity of the anaphase promoting complex/cyclosome (APC/C) conferring substrate specificity upon the complex, thus driving the cell through M phase. It is required for two microtubule-dependent processes, nuclear movement prior to anaphase and chromosome separation.

BUB1 also acts in M phase as a component of the mitotic checkpoint that delays anaphase until all chromosomes are properly attached to the mitotic spindle.

Cyclin A2 - The protein encoded by this gene functions as a regulator of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. It accumulates steadily during G2 and is abruptly destroyed at mitosis. This cyclin binds and activates CDC2 or CDK2 kinases to form a serine/threonine kinase holoenzyme complex imparting substrate specificity to the complex. Thus it is essential for the control of the cell cycle at the G1/S (start) and the G2/M (mitosis) transitions.

Cyclin B2 - The protein encoded by this gene also functions as a regulator of CDK kinases. Again, different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. It accumulates steadily during G2 and is abruptly destroyed at mitosis. It is essential for the control of the cell cycle at the G2/M (mitosis) transition.

Interacts with the CDC2 protein kinase to form a serine/threonine kinase holoenzyme complex also known as maturation promoting factor (MPF). The cyclin subunit imparts substrate specificity to the complex,

Also interacts with transforming growth factor beta RII. Thus, cyclin B2/cdc2 plays a key role in transforming growth factor beta-mediated cell cycle control.

UPREGULATED GENES – Tissue Remodeling

A second system affected by the extract is that of eicosanoid metabolism (2 significant genes). Both are receptors for PGE2. Only four receptors exist to accept and react with PGE2, lending significance to the effect. Below is a summary of PGE2 receptor and PGE2 effects.

Receptor for prostaglandin E2 (PGE2). The protein encoded by this gene is a member of the G-protein coupled receptor family. The activity of this membrane bound receptor is mediated by G(s) proteins that stimulate Adenylate cyclase. This protein is one of only four receptors identified for prostaglandin E2 (PGE2). It has been shown to mediate PGE2 induced expression of early growth response 1 (EGR1), regulate the level and stability of cyclooxygenase-2 mRNA, and lead to the phosphorylation of glycogen synthase kinase-3. Knockout studies in mice suggest that this receptor may be involved in the initiation of skin immune responses. Has a relaxing effect on smooth muscle.

UPREGULATED GENES – ANTI-APOPTOSIS

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A third system affected was that of apoptosis, alleviated by the up-regulation of MgSOD. A summary of the activity of this gene is summarized below.

MgSOD is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein that forms a homotetramer and binds one manganese ion per subunit. This protein binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen.

The human MgSOD gene has a typical housekeeping gene promoter, but is highly inducible by various physical, chemical, and biological agents via the activity of the transcription factor SP-1. An enhancer element is found in the promoter region of the human MgSOD gene. Several important enhancer elements are located in the second intron. The NF-kappa B site in the second intron is essential but not sufficient for high-level induction of MgSOD by cytokines.

Reactive oxygen species are required for cell proliferation but can also induce apoptosis. In proliferating cells this paradox is solved by the activation of protein kinase B (PKB; also known as c-Akt), which protects proliferating cells from apoptosis. By contrast, in quiescent cells (that lack PKB activity) an alternative mechanism is induced as a consequence of PKB inactivity. This mechanism entails the activation of Forkhead transcription factors, the direct transcriptional activation of MgSOD and the subsequent reduction of reactive oxygen species. Increased resistance to oxidative stress is associated with longevity. The model of Forkhead involvement in regulating longevity stems from genetic analysis in *Caenorhabditis elegans*, and it has been concluded that this model also extends to mammalian systems. It has recently been concluded that the induction of this enzyme decreases with aging.

DOWNREGULATED GENES –

TNF-related apoptosis-inducing ligand (TRAIL, also called Apo2L), a novel member of TNF super family that induces apoptosis in transformed cell lines of diverse origin, was significantly downregulated. Skin expresses a functional form of TRAIL.

Cell communication was also down-regulated lending one to consider the proliferative status of the tissue. As proliferating cells pass through the cell cycle, cell communication is generally down-regulated as cell prepares to divide.

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Human gene expression via microarray method has demonstrated the many functional benefits DC Upregulex can offer for skin care applications. DC Upregulex can support anti-aging claims, condition dry fragile skin and help prevent stretch marks via multiple activities we describe as skin nutrition. DC Upregulex offers a unique INCI name listed with the CTFA as: Water (and) Butylene Glycol (and) Phospholipids (and) Hydrolyzed Sericin.