Research Overview for Health Professionals

Oralcell -A Regenerative Bioceutical Complex

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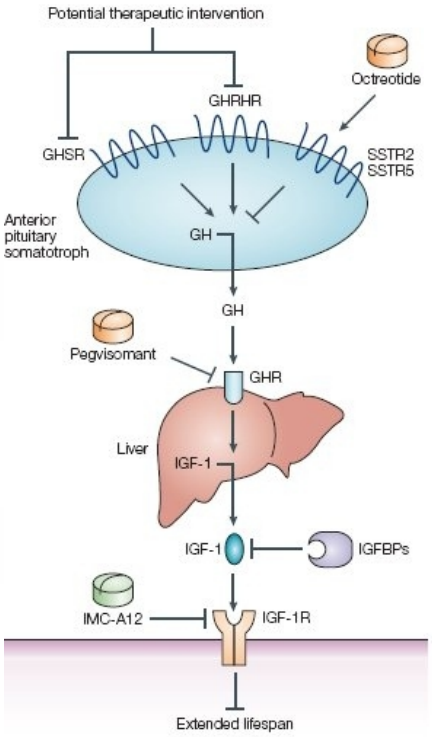
Stem Cell Overview-The Future of Regenerative Medicine

The recent scientific breakthroughs that allowed the cloning of mammals from differentiated cells have refuted the old dogma that development is an irreversible process. Modern science has demonstrated that the DNA in an adult nucleus can be reprogrammed into an embryonic state that can direct the complete development of a new organism (1). Specific regulatory factors in stem cells enhance the regenerative capacity by introducing cellular de-differentiation of adult cells (2). The activation of quiescent stem cells in adult tissues – from amphibians to humans – provides a pool of cells for continual maintenance and repair of the postnatal organism after birth (3).

The ultimate goal of regenerative medicine is to extend longevity and quality of life. Studies in a variety of species demonstrate that caloric restriction is the most effective lifestyle change to extend lifespan. Recently, numerous genes have been identified that either enhance or shorter longevity (4,5). The challenge for the field of anti-aging medicine is to identify methods to modulate the activity of the most important molecular targets to enhance longevity. Novavit Complex represents an innovative holistic approach to biotechnology using embryonic cells that exemplifies the quote of Hippocrates, "let thy food be thy medicine and let thy medicine be thy food."

Human Growth Hormone Therapy-The Wrong Target?

The well documented decline in human growth hormone (GH) levels during aging (somatopause) has resulted in the popularity of replacement therapyin anti-aging clinics. Overall, clinical studies of GH therapy in patients with GH deficiency demonstrate the main benefits are increased lean body mass and bone mineral density (6,7). Despite these benefits on the quality of life, numerous studies indicate that GH actually decreases longevity in animals and centarians (8,9). For example, long-term treatment of obese rats with GH reduced lifespan (10). Lifelong absence of GH in knockout mice results in a 20-70% enhanced lifespan that could not be extended further by caloric restriction (11,12). The GH deficient mice exhibit increased insulin sensitivity and glucose homeostasis that promote longevity (13,14). The decline of GH during aging in mice has been shown to reduce neoplastic disease, age-related pathologies, and to increase lifespan (15). Furthermore, a mutation in the transcription factor Pit-1 decreases GH levels and increases the resistance to oxidative stress enhances the lifespan of mice(16,17). Taken together, the studies indicate that GH therapy could have a negative effect on lifespan in humans and that replacement therapy should not exceed the age-related reference range.



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The Insulin-Like Growth Factor Longevity Pathway- Klotho

The key conserved pathway (from yeast to humans) that has been shown to regulate lifespan is blockade of insulin-like growth factor 1 (IGF1) signaling(18). In contrast to the negative effects of GH on longevity, several genes in the IGF1 signaling pathway have been recently identified that extend the lifespan of mice. The common functions of these genes relate totheir effects on the caloric restriction pathway controlling insulin sensitivity and the regulation of resistance to oxidative stress (19). Selective inhibition of the IGF1 signaling pathway will represent a breakthrough in anti-aging medicine.

Recent studies have identified a peptide hormone called klotho that enhances longevity by blocking both IFG1 signaling and inhibiting GH levels (20). Absence of the klotho gene in mice causes premature aging that increases cardiovascular disease, osteoporosis, skin atrophy, pulmonary emphysema, immune function, and cognitive impairment (21-25). Furthermore, polymorphisms in the human klotho gene are associated with decreased lifespan (26).

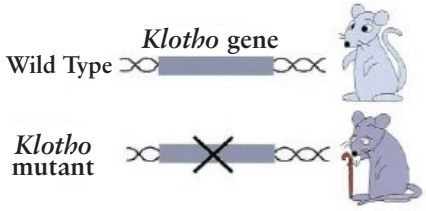

Fig. 1. A mutant model mouse is useful for studies of aging. The *klotho* phenotype (premature aging) is caused by a disruption of the single gene, *klotho*.

Increased expression of klotho has been found to extends the lifespan of mice by inhibiting the signaling of insulin and IGF-1 (21). The klotho hormone has been shown to increase the expression of manganese superoxide dismutase that it turn facilitates removal of reactive oxidative species and confers oxidative stress resistance (27).

Stem Cells Programmed for Unlimited Longevity –A Factory for Anti-Aging Biomolecules

In contrast to adult cells that utilize GH to stimulate growth, stem cells require klotho, leukemia inhibitory factor, cripto and many other embryonic growth factors (28,29). Proteomic studies have identified many unique growth factors and matrix proteins that specifically regulate the growth, metabolism, and signal transduction of embryonic stem cells (30,31). For example, cripto, or TDGF1, is an autocrine stem cell growth factor that is required for embryogenesis by stimulating stem cell proliferation at the expense of differentiation (32).

Recent studies have found that pluripotent stem cells require a set of genes that are not expressed in other cells types (33-35). A common subset of at least 92 evolutionarily conserved regulatory genes (e.g. nanog, oct-4, sox-2) provide a unique molecular signature that are responsible for the pluripotent capacity of avian, mouse, and human stem cells (36,37). The expression of these genes, together with the absence of differentiation markers, constitutes a signature profile of undifferentiated stem cells irrespective of their species of origin (38)(40-44). These genes are involved with extracellular matrix, apoptosis, metabolism and other cellular functions are expressed in avian, murine, and human stem cells.

For example, a recent patent disclosed isolating phospholipids from 6-14 day old chick embryos extended the lifespan of mice. Changes in the composition of phospholipids in the chicken and duck extracts contain alkenyl and acyl groups that are not typically present in later stages of development and were found to extend the live of mice and to reverse several age related dysfunctions in human subjects 47 to 70 years old (39-43). Pluripotent stem cells have also been found to express proteoglycans with specific glycoprotein modifications(44-47). For example, chondroitin sulphate and dermatan sulphate in early chick embryos express unique proteoglycan modifications that are not expressed in later stages of development (48). Thus, no single gene is responsible for the undifferentiated state of stems cells. Rather, stem cells are compose d of hundreds to thousands unique bioactive molecules that can effect the function of the adult organism.

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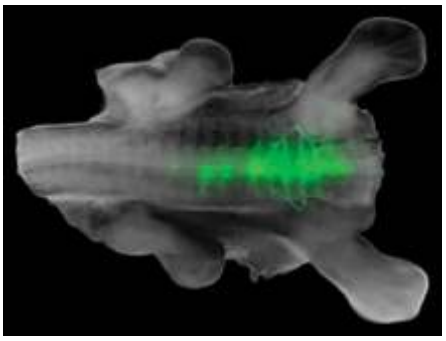
Senescence and Oxidative Stress- Reversal by Stem Cell Biomolecules

Recent studies have shown that aging results in fibroblast senescence that induces alterations in oxidative stress pathways and tissue repair mechanisms (i.e. matrix metalloproteases) leading to loss of tissue function and organization that is a hallmark of aging (49,50). The growth of senescent fibroblasts has been shown to be restored by a transient exposure to an embryonic cell extract (51). klotho is a pluripotent stem cell maintenance factor that has been shown to prevents apoptosis and senescence of differentiated cells (52-54). Recent studies show that extracts from embryonic stem cells reprogram differentiated cells into multipotent or pluripotent stem cells(55-57). Asoluble factor has been also been identified that triggers cell cycle re-entry in differentiated muscle cells(58).

Stem Cell Embryogenesis is Conserved Between Species!!!



Despite the genetic differences between ducks and humans(59), extensive research using avian embryos demonstrates that biological function of the key regulatory factors for embryonic development are evolutionarily conserved. For example, a recent study found that when human hematopoetic stem cells (HSC) from adult bone marrow were implanted into lesions of the developing spinal cord in chick embryo that the factors present in the microenvironment of the chick (i.e. growth factors, matrix) can stimulate adult human HSC to differentiate into full-fledged neurons (60). Furthermore, other studies have shown that human embryonic stem (ES) cells, rat mesenchymal stem cells, and mouse neuronal stem cells (**green cells in picture**) can all integrate into the chicken embryo and differentiate into various cell types(61-63). Furthermore, avian growth factors can directly stimulate the growth of mouse ES cells in cell culture (64). Avian embryonic stem cells do not express GH, but express the avian genes encoding klotho (65). The lack of species specificity for morphogenic factors is further demonstrated by the observations that soluble factors from the newt can restore the endogenous regenerative capacity of differentiated mammalian cells (66,67).

Stem Cell Biology -Changes Induced by Cell Culture

Cell therapy is one of the most exciting fields in translational medicine. It stands at the intersection of a variety of rapidly developing scientific disciplines: stem cell biology, immunology, tissue engineering, molecular biology, regenerative medicine, and clinical research. Although single recombinant protein therapies have been developed to treat specific diseases (e.g. insulin, erythropoietin), most common diseases processes are not due to a deficiency in a single protein but develop due to alterations in the complex interactions of a variety of cell components. While human stem cell therapy using live cells is a promising approach, current ethical/regulatory guidelines and potential safety/efficacy issues do not allow the use of human stem cells in the USA.

Stem cell extracts represent an alternative solution to deliver the multitude of growth regulatory factors to promote longevity. Stem cells extracts could be derived from dissected embryos or from cell lines propagated in cell culture. The embryonic genes have distinct special and temporal expression profiles indicating that stem cells harvested at different times of development will contain different biomolecules (68). A recent study of purified uncultur ed stem cells found significant alterations in gene expression after *in vitro* cell culture. In cell culture, transcripts associated with cell cycle, stemness, certain cytokines and organ specific genes were downregulated, whereas transcripts associated with signal transduction, cell adhesion and cytoskeletal proteins were upregulated (60). These changes result from the consequence of *in vitro* cell propagation on a plastic surface with inappropriate growth factors that do not mimic the microenvironment in the embryo. Thus, freshly dissected embryonic tissues at specific times in embryogenesis are required to produce extracts that

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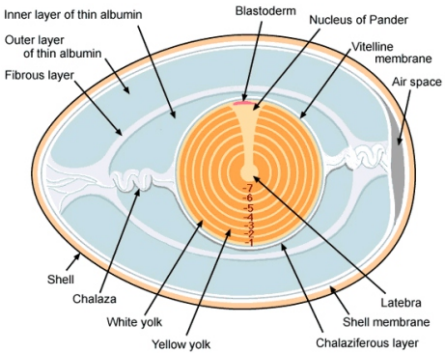
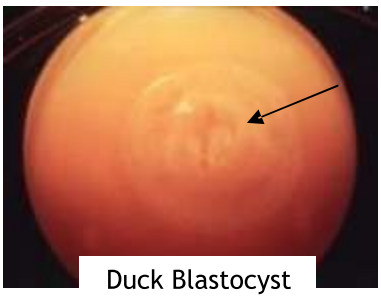
Oralcell The goal of Oralcell was to create the world's first authentic embryonic cell bioceutical. The scientists and eng ineers at Oralcell needed to do the impossible because the number of stem cells is very limited in any developing organism that all start at the same place – a sperm and an oocyte. Avian cells were chosen as the optimal source due to the ability to microdissect blastoderm cells from millions of eggs to create an authentic stem cell bioceutical. Each vial of Oralcell contains blastoderm stem cells micro-dissected from a lots of eggs using proprietary process.



While chicken eggs would have been easier to obtain, Oralcell chose to use Peking Duck eggs harvested using USDA approved procedures. The confined wire cage propagation methods used to produce commercial chickens include many hormones and vaccinations that significantly alter the biochemistry of the chicken eggs and poultry meat (69,70). In contrast, the free-range unvaccinated Peking Ducks used to create Oralcell. The extract is freeze dried using a proprietary process and the product has been certified by an independent laboratory to be free of salmonella, E. coli and other potential biological contaminants.


Duck Blastocyst

The gestation period for the development of duckling takes 27-28 days after fertilization. Interestingly, in several Asian countries live duck embryos harvested between 16-18 days represent a common health tonic called Balut (Philippines), embryonated egg (China), or hot vit lon (Vietnamese). Balut would not be too palatable for most Americans because day 16-18 embryos have already produced bones Pluripotent stem cells are micro-dissectedfrom duck blastocysts from early embryos harvested at a proprietary time less than one day following fertilization. Studies show that these stem cells are capable of proliferation and self-renewal and have the capacity to differentiate into all somatic cell types (71). Similar to human cells, avian embryonic stem cells express telomerase to endure multiple rounds of cell division and that differentiated avian cells down-regulate telomerase expression coincident with organogenesis and somatic differentiation(72).

Activity of Cell Extracts- Hundreds of Research Studies

The biological activity of a variety of tissue extracts has been demonstrated in hundreds of studies in humans and animal models of disease. Fo r example, oral bovine thymus has been shown to exert corrective actions on mouse and dog age-related disorders (73). The clinical benefit of oral spleen extracts have been demonstrated in gastroduodenal ulcers (74). Embryonic peptides have been shown to lower cardiac risk factors including LDL-cholesterol, apolipoproteins A/B, and insulin levels in 40 subjects aged 50-75 years old (75). Hundreds of research publications have documented the biological activity of animal tissue extracts from spleen, liver, and adrenal glands (76-78) Solcoseryl is an extract derived from calf blood that is widely used outside the United States for a variety of health conditions (79). A human placenta total lipid extract containing sphingolipids has been shown to stimulate melanogenesis and pigmentation in mice (80,81). A small peptide has been purified from placenta extract with homology to fibronectin type III that has activity in wound healing (81). Pig placenta extract has been found to modulate the immunity in mouse and human lymphocytes (82). A human fetal cell extract was found to inhibit micronucleus formation induced by carcinogens indicating factors exist in fetal extracts that exhibit anti-mutagenic effects(83)

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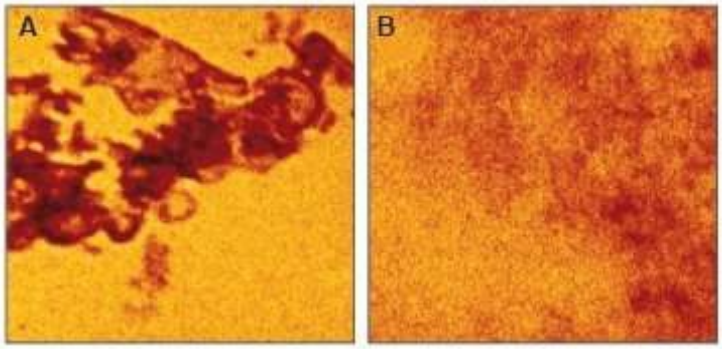
The Oralcell Complex –Enhancing Stem Cell Extract Bioavailablity

The Oralcell Complex has been created using a proprietary form of an organic polyelectrolyte (i.e. montmorillonite) that contains high levels of humic and fulvic acids. This natural material has been shown to have very unique properties that will protect the stem cell extract from gastrointestinal degradation and facilitates uptake of active biomolecules in the small intestine. Safety studies have demonstrated that montmorillonite clay (3gms/day) does not induce any changes in the hematology, liver and kidney function(84).



A study published in *Science*, demonstrated that fulvic acid forms macromolecular structures (i.e. coils, Figure A) at low pH that disperse (Figure B) at high pH (85). The macromolecular structures protect bound molecules from proteolytic and acid damage in the stomach. The binding of proteins to montmorillonite have been shown to exhibit pH dependence of hydrophobic, hydrophilic, and electrostatic interactions. At acidic pH (e.g. stomach acid), montmorillonite is in a flocculated state and the rate of dispersion of bound molecules is inhibited. Upon increasing the pH to 7 (e.g. small intestine), the clay particles progressively deflocculate and the rate of release of bound molecules increases (86). The cellular uptake of various drugs was increased using montmorillonite nanoparticles (87). Furthermore, montmorillonite was found to provide a higher level of protection of DNA against degradation by DNAase (88). Oral administration of DNA complexed to montmorillonite provided protection from the acid environment of the stomach and DNA degrading enzymes in the intestine, and successfully delivered the plasmid DNA into cells of the mouse small intestine(89).

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