Research Overview for Health Professionals

Novavit –A Regenerative Bioceutical Complex

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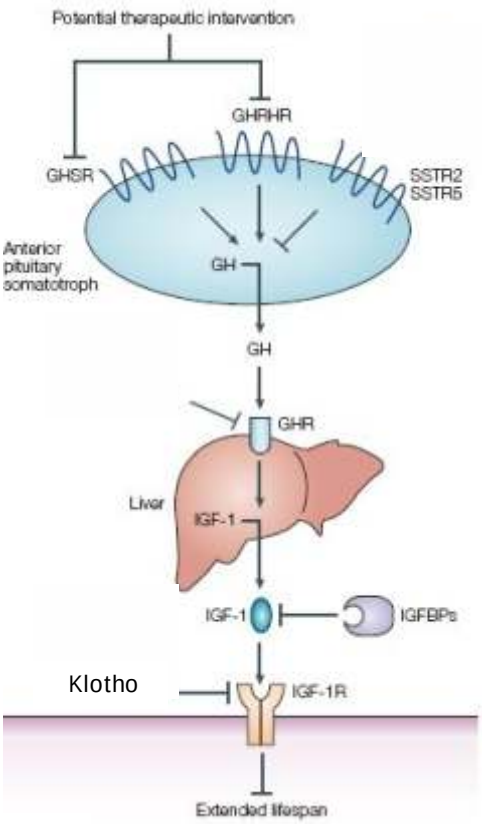
Stem Cell Overview–The Future of Regenerative Medicine

The recent scientific breakthroughs that allowed the cloning of mammals from differentiated cells have refuted the old dogma that development is an ir reversible process. Modern science has demonstrated that the DNA in an adult nucleus can be reprogram med 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 adu lt 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 enhanc e or shorter longevity (4,5). The challenge for the f ield 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 exemp lifies 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 documente d 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 patient s with GH deficiency demonstrate the main benefits are increased lean body mass and bone mine ral 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.



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 oxidati ve 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 agin g 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).

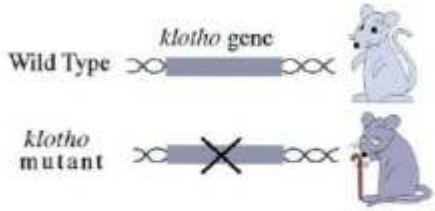


Fig. 1. A mutant model mouse is useful for studies of aging. The *klotho* phenotype (premature aging) is caused by a disruption of the single gene, *klotho*.

Increased expression of klotho has be en 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 expressio n 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 growt h 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.

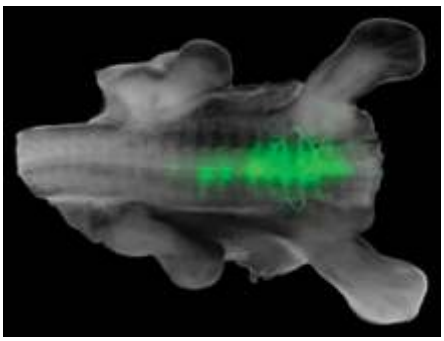
For example, a recent patent disclosed isolating phospholipids from 6-14 day old chick embryos extended the lifespan of mice. Changes in the composition of phospholipids in the chicken and duck extracts contain alkenyl and acyl groups that are not typically present in later stages of development and 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 proteoglyca n 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.

Complex Novavit

Senectud y Stress Oxidativo Revertido por Biomoléculas de Células Madres

Los estudios recientes han mostrado que los resultados del envejecimiento en la senectud fibroplástica que induce a alteraciones en los caminos del stress oxidativo y mecanismos reparadores de tejidos (p.ej: matriz metaproteasa), llevan a la pérdida de la función de los tejidos y la organización que es un sello de envejecimiento (49,50). El crecimiento de la senectud fibroplástica ha demostrado ser restauradora de una exposición de transición a un extracto de célula embrionaria (51). El klotho es un factor de mantenimiento pluripotencial de célula madre que ha sido mostrado para prevenir la apoptosis y la senectud de las células diferenciadas (52-54). Los estudios recientes muestran que los extractos de células embrionarias reprogramen células diferenciadas en multipotentes o células madres pluripoteneciales (55-57). Un factor soluble también ha sido identificado como que provoca el ciclo de reingreso las células en células de músculo diferenciadas (58).

Las Células Madres son Conservadas entre Especies!!!



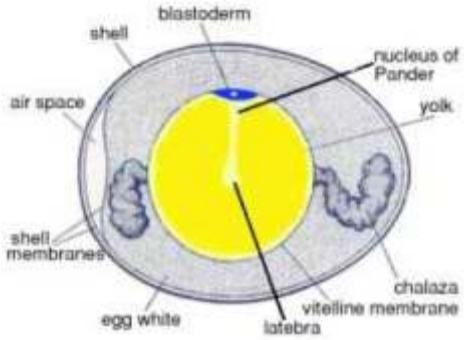
A pesar de las diferencias genéticas entre patos y humanos (59), extensas investigaciones usando embriones de ave, demuestran que la función biológica de los factores reguladores claves para el desarrollo embrionario son evolutivamente conservados. Por ejemplo, un estudio reciente encontró que cuando las células madres hematopoyéticas en humanos (HSC) en la medula ósea de adultos, fueron implan tadas en lesiones de la espina en desarrollo en un embrión de pollito, los factores presentes en el micro ambiente del pollito (factores de crecimiento, matriz) pueden estimular el HSC en humanos adultos para diferenciar entre las neuronas maduras (60). Además otros estudios han demostrado que las células madres embrionarias humanas (ES), células madres mesenquimáticas de rata y células madres neurológicas de ratones (células verdes en el cuadro) todas pueden integrarse en el embrión de pollo y diferenciarse en varios tipos de células (61-63). Además, los factores de crecimiento de aves pueden estimular directamente el crecimiento de células de ratones ES en la cultura celular (64). Las células madres embrionarias en aves no expresan GH, pero expresan los genes aviarios que codifican el klotho (65). La carencia de especies específicamente para factores morfogénicos han demostrado por las observaciones que los factores solubles del newt puede restaurar la capacidad regeneradora endógena de células mamíferas diferenciadas (66,67).

Biología de la células madres - Cambios Inducidos por la Cultura Celular

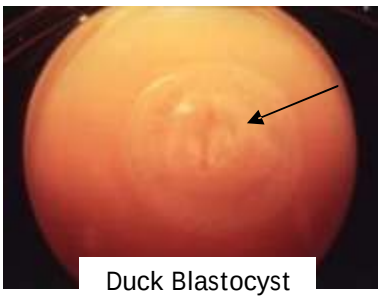
La Terapia Celular es uno de los campos más interesantes en la medicina de translación. Esto se basa en la intersección de una variedad de rápidos desarrollos de disciplinas científicas: Biología de células madres, inmunología, ingeniería de tejidos, biología molecular, medicina regenerativa e investigación clínica. Aunque las terapias individuales de recombinar proteínas han sido desarrolladas para tratar enfermedades específicas (p.ej: insulina, eritropoyetina), los procesos de enfermedades más comunes no son debido a una deficiencia en una sola proteína, pero se desarrolla debido a alteraciones en las interacciones complejas de una variedad de componentes de célula. Mientras las terapias de células madres de humanos usan células vivas para un acercamiento prometedor, las pautas éticas- reguladoras corrientes y las cuestiones de seguridad-eficacia potenciales no permiten el uso de células madres humanas en los EE. UU.

Los extractos de célula madres representan una solución alternativa de entregar una multitud de factores reguladores del crecimiento para promover longevidad. Los extractos de células madre podrían ser sacados de embriones disectados o de las líneas de la célula propagadas en cultivos celulares. Los genes embrionarios tienen distinción especial y perfil de expresión temporal indicando que las células madres cosechadas en tiempos diferentes del desarrollo tendrán diferente biomoléculas (68).Un estudio reciente de células madres no cultivadas purificadas encontró significativas alteraciones en la expresión de genes después de un cultivo de célula en vitro. En el cultivo celular, las transcripciones asociadas con el ciclo de la célula, la falta de soporte, cierto citokinas y genes específicos en órganos fueron regulados a un bajo nivel mientras que transcripciones asociadas con la señal de transducción, la adherencia de célula y las proteínas cytoskeletal, fueron reguladas a un nivel mas alto (60). Estos cambios resultan como consecuencia de la propagación de la célula en vitro en una superficie plástica con factores de crecimiento inadecuados que no imitan el micro ambiente del embrión. Así, tejidos embrionarios frescos disectados en tiempos específicos de embriogenesis son requeridos para producir extractos que representen el autentico perfil de biomoleculas de células madres.

Complex Novavit The goal of Novavit was to create the world's first authentic embryonic cell bioceutical. The scientists and eng ineers at Novavit 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 micro-dissect blastoderm cells from millions of eggs to create an authentic stem cell bioceutical . Each vial of Novavi t contains blastoderm stem cells micro-dissected from 21 eggs (625 eggs for a 30 day supply) using a proprietary process.



While chicken eggs would have been easier to obtain, Novavit chose to use Peking Duck eggs harvested using USDA approved pro cedures. 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 Novavit. The extract is freeze dried using a proprietary proces s and the product has been certified by an indepen dent laboratory to be free of salmonel la, 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 (P hilippines), 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 somati c 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).

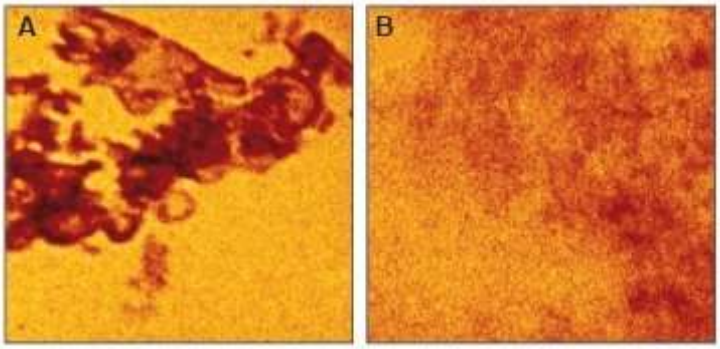
Activity of Cell Extracts– Hundreds of Research Studies

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 thym us 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 contai ning sphingolipids has been shown to stimulate melanogenesis and pigmentation in mice (80,8 1). 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)

Complex
Novavit

The Novavit Complex –Enhancing Stem Cell Extract Bioavailablity

The Novavit 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 intesti ne. 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 dependenc e 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 progressi vely 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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