

LE Magazine March 2002

## ABSTRACTS

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## Melatonin

Homeostatic versus circadian effects of melatonin on core body temperature in humans.

Evidence obtained in animals has suggested a link of the pineal gland and its hormone melatonin with the regulation of core body temperature (CBT). Depending on the species considered, melatonin intervenes in generating seasonal rhythms of daily torpor and hibernation, in heat stress tolerance, and in setting the CBT set point. In humans, the circadian rhythms of melatonin is strictly associated with that of CBT, the nocturnal decline of CBT being inversely related to the rise of melatonin. Whereas there is inconsistent evidence for the suggestion that the decline of CBT may prompt the release of melatonin, conversely, stringent data indicate that melatonin decreases CBT. Administration of melatonin during the day, when it is not normally secreted, decreases CBT by about 0.3 to 0.4 degree C, and suppression of melatonin at night enhances CBT by about the same magnitude. Accordingly, the nocturnal rise of melatonin contributes to the circadian amplitude of CBT. The mechanisms through which melatonin decreases CBT are unclear. It is known that melatonin enhances heat loss, but a reduction of heat production cannot be excluded. Besides actions on peripheral vessels aimed to favor heat loss, it is likely that the effect of melatonin to reduce CBT is exerted mainly in the hypothalamus, where thermoregulatory centers are located. Recent observations have shown that the acute thermoregulatory effects induced by melatonin and bright light are independent of their circadian phase-shifting effects. The effect of melatonin ultimately brings a saving of energy and is reduced in at least two physiological situations: aging and the luteal menstrual phase. In both conditions, melatonin does not exert its CBT-lowering effects. Whereas in older women this effect may represent an age-related alteration, in the luteal phase this modification may represent a mechanism of keeping CBT higher at night to promote a better embryo implantation and survival.

J Biol Rhythms 1997 Dec;12(6):509-17

## Melatonin and sleep in humans.

Early studies on the physiological effects of melatonin typically reported hypnotic 'side-effects'. Later studies, specifically addressing this action, failed to reliably replicate hypnotic effects using standard polysomnography. This difference may be related to differences in the basic physiological action of melatonin compared with more conventional hypnotics. It is suggested that melatonin exerts a hypnotic effect through thermoregulatory mechanisms. By lowering core body temperature, melatonin reduces arousal and increases sleep-propensity. Thus, in humans, one role of melatonin is to transduce the light-dark cycle and define a window-of-opportunity in which sleep-propensity is enhanced. As such, melatonin is likely to be an effective hypnotic agent for sleep disruption associated with elevated temperature due to low circulating melatonin levels. The combined circadian and hypnotic effects of melatonin suggest a synergistic action in the treatment of sleep disorders related to the inappropriate timing of sleep and wakefulness. Adjuvant melatonin may also improve sleep disruption caused by drugs known to alter normal melatonin production (e.g., beta-blockers and benzodiazepines). If melatonin is to be developed as a successful clinical treatment, differences between the pharmacological profile following exogenous administration and the normal endogenous rhythm should be minimized. Continued development as a useful clinical tool requires control of both the amplitude and duration of the exogenous melatonin pulse. There is a need to develop novel drug delivery systems that can reliably produce a square-wave pulse of melatonin at physiological levels for 8 to 10 hr duration.

J Pineal Res 1993 Aug;15(1):1-12

## Melatonin therapy of advanced human malignant melanoma.

We undertook a study to investigate the therapeutic potential of orally administered melatonin in patients with advanced melanoma. Forty-two patients received melatonin in doses ranging from 5 mg/m<sup>2</sup>/day to 700 mg/m<sup>2</sup>/day in four divided doses. Two were excluded from analysis. After a median follow-up of 5 weeks, six patients had partial responses, six additional patients had stable disease. Sites of response included the central nervous system, subcutaneous tissue and lung. The median response duration was 33 weeks for the partial responders. There was a suggestion of a dose-response relationship. The toxicity encountered was minimal and consisted primarily of fatigue in 17 of 40 patients. Melatonin also appeared to reduce basal levels of

follicle-stimulating hormone (FSH). No significant changes in serum levels of luteinizing hormone (LH) or thyroid stimulating hormone (TSH). We conclude that further study of melatonin as a potentially useful agent in metastatic melanoma is warranted.

Melanoma Res 1991 Nov-Dec;1(4):237-43

Artificial life extension. The epigenetic approach.

An epigenetic approach starts out from the direct (rather than the underlying genetic) causes. An epigenetic approach to aging has little chance of succeeding before a minimum amount of knowledge has been accumulated on the "genetic programming" that is currently believed to underlie aging. Two recent advances, one empirical and one theoretical, jointly brighten the prospect. The empirical one is the discovery that melatonin functions as an aging-controlling hormone in mammals. In 1979, Dilman and co-workers isolated a biologically active pineal extract (epithalamin) in rats which, as they later showed, stimulates melatonin production. Pierpaoli and co-workers in 1987 directly administered melatonin to mice. Both groups observed a surprising 25-percent increase of life span in conjunction with a postponed senescence. A similar effect was also achieved with an engraftment of young pineal tissue into the thymus of old mice by Pierpaoli's group. Beneficial effects of epithalamin in humans were reported by Dilman's group. The second advance is a deductive evolution-theoretical approach to aging discovered in 1988. In populations living in a niche with a fixed carrying capacity, any individual is in the long run replaced by a single successor. It follows that, as the expected cumulative number of adult progeny of the same sex approaches unity as a function of life time of the progenitor, the latter's survivability must approach zero if the sum is to remain unity. A physiological prediction follows: a centralized physicochemical clock "like a sedimentation process" must exist somewhere in the organism controlling a secreted substance that reaches all cells. In this way, the pineal coacervates and the pineal's hormonal product melatonin were arrived at on an independent route again. While melatonin as a drug has been used on human volunteers for decades, its anti-aging effect has yet to be proved. Detailed hormone profiles in different age groups and under different life styles have to be performed. A modified Hayflick in vitro experiment is also needed to elucidate the mechanism by which melatonin works in cells.

Ann N Y Acad Sci 1994 May 31;719:474-82

Randomized study with the pineal hormone melatonin versus supportive care alone in advanced nonsmall cell lung cancer resistant to a first-line chemotherapy containing cisplatin.

At present, there is no effective medical therapy in metastatic nonsmall cell (NSC) lung cancer patients who progressed under a first-line chemotherapy containing cisplatin. Since recent data have demonstrated the antineoplastic properties and the lack of toxicity of the pineal hormone melatonin (MLT), a randomized study was designed to evaluate the influence of an MLT treatment (10 mg/day orally at 7.00 p.m.) on the survival time at 1 year from the progression under chemotherapy in respect to supportive care alone in a group of metastatic NSC lung cancer patients, who did not respond to a first-line chemotherapy containing cisplatin. The study includes 63 consecutive metastatic NSC lung cancer patients, who were randomized to receive MLT (n = 31) or supportive care alone (n = 32). The percentage of both stabilizations of disease and survival at 1 year was significantly higher in patients treated with MLT than in those treated only with supportive care. No drug-related toxicity was seen in patients treated with MLT, who, on the contrary, showed a significant improvement in performance status. This randomized study shows that the pineal hormone MLT may be successfully administered to prolong the survival time in metastatic NSC lung cancer patients who progressed under a first-line chemotherapy with cisplatin, for whom no other effective therapy is available up to now.

Oncology 1992;49(5):336-9

Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumor patients with poor clinical status.

Melatonin (MLT) has been proven to counteract chemotherapy toxicity, by acting as an anti-oxidant agent, and to promote apoptosis of cancer cells, so enhancing chemotherapy cytotoxicity. The aim of this study was to evaluate the effects of concomitant MLT administration on toxicity and efficacy of several chemotherapeutic combinations in advanced cancer patients with poor clinical status. The study included 250 metastatic solid tumor patients (lung cancer, 104; breast cancer, 77; gastrointestinal tract neoplasms, 42; head and neck cancers, 27), who were randomized to receive MLT (20 mg/day orally every day) plus chemotherapy, or chemotherapy alone. Chemotherapy consisted of cisplatin (CDDP) plus etoposide or gemcitabine alone for lung cancer, doxorubicin alone, mitoxantrone alone or paclitaxel alone for breast cancer, 5-FU plus folinic acid for gastrointestinal tumors and 5-FU plus CDDP for head and neck cancers. The 1-year survival rate and the objective tumor regression rate were significantly higher in patients concomitantly treated with MLT than in those who received chemotherapy (CT) alone (tumor response rate: 42/124 CT + MLT versus 19/126 CT only,  $P < 0.001$ ; 1-year survival: 63/124 CT + MLT versus 29/126 CT only,  $P < 0.001$ ). Moreover, the concomitant administration of MLT significantly reduced the frequency of thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis and asthenia. This study indicates that the pineal hormone MLT may enhance the efficacy of chemotherapy and reduce its toxicity, at least in advanced cancer patients of poor clinical status.

A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state.

Recent studies suggest that the pineal hormone melatonin may reduce chemotherapy-induced immune and bone marrow damage. In addition, melatonin may exert potential oncostatic effects either by stimulating host anticancer immune defenses or by inhibiting tumor growth factor production. On this basis, we have performed a randomized study of chemotherapy alone vs. chemotherapy plus melatonin in advanced non-small cell lung cancer patients (NSCLC) with poor clinical status. The study included 70 consecutive advanced NSCLC patients who were randomized to receive chemotherapy alone with cisplatin (20 mg/m<sup>2</sup>/day i.v. for 3 days) and etoposide (100 mg/m<sup>2</sup>/day i.v. for 3 days) or chemotherapy plus melatonin (20 mg/day orally in the evening). Cycles were repeated at 21-day intervals. Clinical response and toxicity were evaluated according to World Health Organization criteria. A complete response (CR) was achieved in 1/34 patients concomitantly treated with melatonin and in none of the patients receiving chemotherapy alone. Partial response (PR) occurred in 10/34 and in 6/36 patients treated with or without melatonin, respectively. Thus, the tumor response rate was higher in patients receiving melatonin (11/34 vs. 6/36), without, however, statistically significant differences. The percent of 1-year survival was significantly higher in patients treated with melatonin plus chemotherapy than in those who received chemotherapy alone (15/34 vs. 7/36,  $P < 0.05$ ). Finally, chemotherapy was well tolerated in patients receiving melatonin, and in particular the frequency of myelosuppression, neuropathy, and cachexia was significantly lower in the melatonin group. This study shows that the concomitant administration of melatonin may improve the efficacy of chemotherapy, mainly in terms of survival time, and reduce chemotherapeutic toxicity in advanced NSCLC, at least in patients in poor clinical condition.

J Pineal Res 1997 Aug;23(1):15-9

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A randomized study with the pineal hormone melatonin versus supportive care alone in patients with brain metastases due to solid neoplasms.

**BACKGROUND.** Unresectable brain metastases remain an untreatable disease. Because of its antitumor cytostatic action and its anticonvulsant effect, the pineal hormone melatonin could constitute a new effective agent in the treatment of brain metastases. The current study was performed to evaluate the effect of melatonin on the survival time in patients with brain metastases due to solid neoplasms. **METHODS.** The study included 50 patients, who were randomized to be treated with supportive care alone (steroids plus anticonvulsant agents) or with supportive care plus melatonin (20 mg/day at 8:00 p.m. orally). **RESULTS.** The survival at 1 year, free-from-brain-progression period, and mean survival time were significantly higher in patients treated with melatonin than in those who received the supportive care alone. Conversely, steroid-induced metabolic and infective complications were significantly more frequent in patients treated with supportive care alone than in those concomitantly treated with melatonin. **CONCLUSIONS.** The pineal hormone melatonin may be able to improve the survival time and the quality of life in patients with brain metastases due to solid tumors.

Cancer 1994 Feb 1;73(3):699-701

Modulation of cancer endocrine therapy by melatonin: a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone.

Recent observations have shown that the pineal hormone melatonin (MLT) may modulate oestrogen receptor (ER) expression and inhibit breast cancer cell growth. On this basis, we have evaluated the biological and clinical effects of a concomitant MLT therapy in women with metastatic breast cancer who had progressed in response to tamoxifen (TMX) alone. The study included 14 patients with metastasis who did not respond ( $n = 3$ ) to therapy with TMX alone or progressed after initial stable disease (SD) ( $n = 11$ ). MLT was given orally at 20 mg day<sup>-1</sup> in the evening, every day starting 7 days before TMX, which was given orally at 20 mg day<sup>-1</sup> at noon. A partial response was achieved in 4/14 (28.5%) patients (median duration 8 months). The treatment was well tolerated in all cases, and no MLT-induced enhancement of TMX toxicity was seen; on the contrary, most patients experienced a relief of anxiety. Mean serum levels of insulin-like growth factor 1 (IGF-1), which is a growth factor for breast cancer, significantly decreased on therapy, and this decline was significantly higher in responders than in patients with SD or progression. This pilot phase II study would suggest that the concomitant administration of the pineal hormone MLT may induce objective tumor regressions in metastatic breast cancer patients refractory to TMX alone.

Br J Cancer 1995 Apr;71(4):854-6

Interleukin-2, melatonin and interleukin-12 as a possible neuroimmune combination in the biotherapy of cancer.

Suppressive events induced by macrophages and TH2 lymphocytes would represent the most important factors responsible for the in vivo reduced efficacy of IL-2 cancer immunotherapy. Previous studies have shown that IL-3 or the pineal hormone MLT may abrogate macrophage-related suppressive events during IL-2 immunotherapy, while TH2-mediated immunosuppression is not neutralized by MLT or IL-3. On the basis of previous experimental data suggesting the inhibitory effect of IL-12 on TH2 activation, this preliminary study has been performed in an attempt to evaluate the influence of IL-12 on TH2 stimulation induced by IL-2 alone or IL-2 plus MLT, by evaluating the release of IL-10, which represents the main suppressive factor produced by TH2 lymphocytes. Pure lymphocyte cultures were incubated for 4 days with IL-2 (100 U/ml), MLT (100 pg/ml), IL-12 (1 ng/ml), IL-2 plus MLT, IL-2 plus IL-12 or IL-2 plus MLT and IL-12. Mean medium concentrations of IL-10 were measured by Elisa. IL-2 alone significantly stimulated IL-10 secretion with respect to the control medium alone, while no difference was observed with MLT alone or IL-12. IL-2-induced stimulation of IL-10 secretion was not abrogated by a concomitant MLT incubation. On the contrary, IL-12 significantly diminished IL-10 release in response to IL-2, and this inhibitory effect was more pronounced when IL-2 was added in association with both IL-12 and MLT. This preliminary study would suggest that the two most important immunosuppressive events occurring during IL-2 therapy, which are mediated by macrophages and TH2-lymphocytes, may be abrogated by a concomitant administration of MLT and IL-12, respectively. Therefore, the association of IL-12 could further amplify IL-2 efficacy with respect to IL-2 alone or IL-2 plus MLT.

J Biol Regul Homeost Agents 1995 Apr-Jun;9(2):63-6

A randomized study with subcutaneous low-dose interleukin 2 alone vs interleukin 2 plus the pineal neurohormone melatonin in

advanced solid neoplasms other than renal cancer and melanoma.

Our previous experimental studies have shown that the best approach to increase the biological anti-tumor activity of interleukin 2 (IL-2) is not co-administration of another cytokine, but the association with immunomodulating neurohormones, in an attempt to reproduce the physiological links between psychoendocrine and immune systems, which play a fundamental role in the regulation of the immune responses. In particular, the association with the pineal neurohormone melatonin (MLT) has been shown to cause tumor regressions in neoplasms that are generally non-responsive to IL-2 alone. To confirm these preliminary results, a clinical trial was performed in locally advanced or metastatic patients with solid tumors other than renal cell cancer and melanoma. The study included 80 consecutive patients, who were randomized to be treated with IL-2 alone subcutaneously (3 million IU day<sup>-1</sup> at 8.00 p.m. 6 days a week for 4 weeks) or IL-2 plus MLT (40 mg day<sup>-1</sup> orally at 8.00 p.m. every day starting 7 days before IL-2). A complete response was obtained in 3/41 patients treated with IL-2 plus MLT and in none of the patients receiving IL-2 alone. A partial response was achieved in 8/41 patients treated with IL-2 plus MLT and in only 1/39 patients treated with IL-2 alone. Tumor objective regression rate was significantly higher in patients treated with IL-2 and MLT than in those receiving IL-2 alone (11/41 vs 1/39,  $P < 0.001$ ). The survival at 1 year was significantly higher in patients treated with IL-2 and MLT than in the IL-2 group (19/41 vs 6/39,  $P < 0.05$ ). Finally, the mean increase in lymphocyte and eosinophil number was significantly higher in the IL-2 plus MLT group than in patients treated with IL-2 alone; on the contrary, the mean increase in the specific marker of macrophage activation neopterin was significantly higher in patients treated with IL-2 alone. The treatment was well tolerated in both groups of patients. This study shows that the concomitant administration of the pineal hormone MLT may increase the efficacy of low-dose IL-2 subcutaneous therapy.

Br J Cancer 1994 Jan;69(1):196-9

Increased survival time in brain glioblastomas by a radioneuroendocrine strategy with radiotherapy plus melatonin compared to radiotherapy alone.

The prognosis of brain glioblastoma is still very poor and the median survival time is generally less than 6 months. At present, no chemotherapy has appeared to influence its prognosis. On the other hand, recent advances in brain tumor biology have suggested that brain tumor growth is at least in part under a neuroendocrine control, mainly realized by opioid peptides and pineal substances. On this basis, we evaluated the influence of a concomitant administration of the pineal hormone melatonin (MLT) in patients with glioblastoma treated with radical or adjuvant radiotherapy (RT). The study included 30 patients with glioblastoma, who were randomized to receive RT alone (60 Gy) or RT plus MLT (20 mg/daily orally) until disease progression. Both the survival curve and the percent of survival at 1 year were significantly higher in patients treated with RT plus MLT than in those receiving RT alone (6/14 vs. 1/16). Moreover, RT or steroid therapy-related toxicities were lower in patients concomitantly treated with MLT. This preliminary study suggests that a radioneuroendocrine approach with RT plus the pineal hormone M.

Oncology 1996 Jan-Feb;53(1):43-6

Is there a role for melatonin in the treatment of neoplastic cachexia?

It is known that neoplastic cachexia shows metabolic characteristics different from other common causes of malnutrition, and that it is mainly due to an abnormal secretion of TNF, whose levels are often high in patients with advanced neoplasia. Previous clinical studies have suggested that the pineal hormone melatonin (MLT), which plays an essential role in the neuroendocrine regulation of biological systems, may improve the clinical status of advanced cancer patients and inhibit TNF secretion. To investigate the relationship between MLT, TNF and cancer-related weight loss, 100 untreatable metastatic solid tumor patients entered this study to receive either supportive care alone, or supportive care plus MLT (20 mg/day orally in the evening). Patients were observed for 3 months, and were considered evaluable when they were observed for at least 2 months. There were 86 evaluable patients, the other 14 patients having died from rapid progression of disease. The per cent of weight loss greater than 10% was significantly higher in patients treated by supportive care alone than in those concomitantly treated by MLT, with no difference in food intake ( $P < 0.01$ ). Mean serum levels of TNF progressively increased in the supportive care group, but to levels that were not significantly different from pretreatment values. In contrast, TNF mean concentrations significantly decreased ( $P < 0.05$ ) in patients concomitantly treated by MLT. These results suggest that the pineal hormone MLT may be effective in the treatment of the neoplastic cachexia by decreasing TNF blood concentrations.

Eur J Cancer 1996 Jul;32A(8):1340-3

The immunoneuroendocrine role of melatonin.

A tight, physiological link between the pineal gland and the immune system is emerging from a series of experimental studies. This link might reflect the evolutionary connection between self-recognition and reproduction. Pinealectomy or other experimental methods which inhibit melatonin synthesis and secretion induce a state of immunodepression which is counteracted by melatonin. In general, melatonin seems to have an immunoenhancing effect that is particularly apparent in immunodepressive states. The negative effect of acute stress or immunosuppressive pharmacological treatments on various immune parameters are counteracted

by melatonin. It seems important to note that one of the main targets of melatonin is the thymus, i.e., the central organ of the immune system. The clinical use of melatonin as an immunotherapeutic agent seems promising in primary and secondary immunodeficiencies as well as in cancer immunotherapy. The immunoenhancing action of melatonin seems to be mediated by T-helper cell-derived opioid peptides as well as by lymphokines and, perhaps, by pituitary hormones. Melatonin-induced-immuno-opioids (MIIO) and lymphokines imply the presence of specific binding sites or melatonin receptors on cells of the immune system. On the other hand, lymphokines such as gamma-interferon and interleukin-2 as well as thymic hormones can modulate the synthesis of melatonin in the pineal gland. The pineal gland might thus be viewed as the crux of a sophisticated immunoneuroendocrine network which functions as an unconscious, diffuse sensory organ.

J Pineal Res 1993 Jan;14(1):1-10

Sleep-inducing effects of low doses of melatonin ingested in the evening.

We previously observed that low oral doses of melatonin given at noon increase blood melatonin concentrations to those normally occurring nocturnally and facilitate sleep onset, as assessed using an involuntary muscle relaxation test. In this study we examined the induction of polysomnographically recorded sleep by similar doses given later in the evening, close to the times of endogenous melatonin release and habitual sleep onset. Volunteers received the hormone (oral doses of 0.3 or 1.0 mg) or placebo at 6, 8 or 9 PM. Latencies to sleep onset, to stage 2 sleep, and to rapid eye movement (REM) sleep were measured polysomnographically. Either dose given at any of the three time points decreased sleep onset latency and latency to stage 2 sleep. Melatonin did not suppress REM sleep or delay its onset. Most volunteers could clearly distinguish between the effects of melatonin and those of placebo when the hormone was tested at 6 or 8 PM. Neither melatonin dose induced "hangover" effects, as assessed with mood and performance tests administered on the morning after treatment. These data provide new evidence that nocturnal melatonin secretion may be involved in physiologic sleep onset and that exogenous melatonin may be useful in treating insomnia.

Clin Pharmacol Ther 1995 May;57(5):552-8

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## ABSTRACTS

### Medical technology

Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells.

Myocyte loss in the ischemically injured mammalian heart often leads to irreversible deficits in cardiac function. To identify a source of stem cells capable of restoring damaged cardiac tissue, we transplanted highly enriched hematopoietic stem cells, the so-called side population (SP) cells, into lethally irradiated mice subsequently rendered ischemic by coronary artery occlusion for 60 minutes followed by reperfusion. The engrafted SP cells (CD34(-)/low, c-Kit(+), Sca-1(+)) or their progeny migrated into ischemic cardiac muscle and blood vessels, differentiated to cardiomyocytes and endothelial cells, and contributed to the formation of functional tissue. SP cells were purified from Rosa26 transgenic mice, which express lacZ widely. Donor-derived cardiomyocytes were found primarily in the peri-infarct region at a prevalence of around 0.02% and were identified by expression of lacZ and alpha-actinin, and lack of expression of CD45. Donor-derived endothelial cells were identified by expression of lacZ and Flt-1, an endothelial marker shown to be absent on SP cells. Endothelial engraftment was found at a prevalence of around 3.3%, primarily in small vessels adjacent to the infarct. Our results demonstrate the cardiomyogenic potential of hematopoietic stem cells and suggest a therapeutic strategy that eventually could benefit patients with myocardial infarction.

J Clin Invest 2001 Jun;107(11):1395-402

Bone marrow cells regenerate infarcted myocardium.

Myocardial infarction leads to loss of tissue and impairment of cardiac performance. The remaining myocytes are unable to reconstitute the necrotic tissue, and the post-infarcted heart deteriorates with time. Injury to a target organ is sensed by distant stem cells, which migrate to the site of damage and undergo alternate stem cell differentiation; these events promote structural and functional repair. This high degree of stem cell plasticity prompted us to test whether dead myocardium could be restored by transplanting bone marrow cells in infarcted mice. We sorted lineage-negative (Lin-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein by fluorescence-activated cell sorting on the basis of c-kit expression. Shortly after coronary ligation, Lin- c-kitPOS cells were injected in the contracting wall bordering the infarct. Here we report that newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. Our studies indicate that locally delivered bone marrow cells can generate de novo myocardium, ameliorating the outcome of coronary artery disease.

Nature 2001 Apr 5;410(6829):701-5

Myocyte transplantation for myocardial repair: a few good cells can mend a broken heart.

Cell transplantation is a potential therapeutic approach for patients with chronic myocardial failure. Experimental transplantation of neonatal and fetal cardiac myocytes showed that the grafted cells can functionally integrate with and augment the function of the recipient heart. Clinical application of this approach will be limited by shortage of donors, chronic rejection, and because it is ethically contentious. By contrast skeletal myoblasts (satellite cells) are abundant and can be grafted successfully into the animal's own heart even after genetic manipulation in vitro. Functional integration of myoblasts, however, is hampered by the lack of intercellular gap junction communication and the difference in excitation-contraction coupling between skeletal and cardiac myocytes. In experimental studies several other cell types have been used to augment cardiac function. In this review we discuss the published results of myocyte transplantation with emphasis on potential sources of cells, the ethics of using donor embryonic and fetal cardiomyocytes, genetic transformation of skeletal myoblasts for myocardial repair, and the functional benefits of cell transplantation to the failing heart.

Ann Thorac Surg 2001 May;71(5):1724-33

Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells in vivo.

The versatility of stem cells has only recently been fully recognized. There is evidence that upon adoptive bone marrow (BM) transplantation (BMT), donor-derived cells can give rise to neuronal phenotypes in the brains of recipient mice. Yet only few cells with the characteristic shape of neurons were detected 1-6 mo post-BMT using transgenic or newborn mutant mice. To evaluate

the potential of BM to generate mature neurons in adult C57BL/6 mice, we transferred the enhanced green fluorescent protein (GFP) gene into BM cells using a murine stem cell virus-based retroviral vector. Stable and high level long-term GFP expression was observed in mice transplanted with the transduced BM. Engraftment of GFP-expressing cells in the brain was monitored by intravital microscopy. In a long-term follow up of 15 mo post-BMT, fully developed Purkinje neurons were found to express GFP in both cerebellar hemispheres and in all chimeric mice. GFP-positive Purkinje cells were also detected in BM chimeras from transgenic mice that ubiquitously express GFP. Based on morphologic criteria and the expression of glutamic acid decarboxylase, the newly generated Purkinje cells were functional.

Journal of Cell Biology 2001, 155:5:733-738, Nov. 26

Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell.

Purification of rare hematopoietic stem cell(s) (HSC) to homogeneity is required to study their self-renewal, differentiation, phenotype, and homing. Long-term repopulation (LTR) of irradiated hosts and serial transplantation to secondary hosts represent the gold standard for demonstrating self-renewal and differentiation, the defining properties of HSC. We show that rare cells that home to bone marrow can LTR primary and secondary recipients. During the homing, CD34 and SCA-1 expression increases uniquely on cells that home to marrow. These adult bone marrow cells have tremendous differentiative capacity as they can also differentiate into epithelial cells of the liver, lung, GI tract and skin. This finding may contribute to clinical treatment of genetic disease or tissue repair.

Cell 2001 May 4;105(3):369-77

Embryonic stem cell lines derived from human blastocysts.

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle and striated muscle (mesoderm); and neural epithelium, embryonic ganglia and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery and transplantation medicine.

Science 1998 Nov 6;282(5391):1145-7

Neural progenitors from human embryonic stem cells.

The derivation of neural progenitor cells from human embryonic stem (ES) cells is of value both in the study of early human neurogenesis and in the creation of an unlimited source of donor cells for neural transplantation therapy. Here we report the generation of enriched and expandable preparations of proliferating neural progenitors from human ES cells. The neural progenitors could differentiate in vitro into the three neural lineages?astrocytes, oligodendrocytes and mature neurons. When human neural progenitors were transplanted into the ventricles of newborn mouse brains, they incorporated in large numbers into the host brain parenchyma, demonstrated widespread distribution and differentiated into progeny of the three neural lineages. The transplanted cells migrated along established brain migratory tracks in the host brain and differentiated in a region-specific manner, indicating that they could respond to local cues and participate in the processes of host brain development. Our observations set the stage for future developments that may allow the use of human ES cells for the treatment of neurological disorders.

Nat Biotechnol 2001 Dec;19(12):1134-40

In vitro differentiation of transplantable neural precursors from human embryonic stem cells.

The remarkable developmental potential and replicative capacity of human embryonic stem (ES) cells promise an almost unlimited supply of specific cell types for transplantation therapies. Here we describe the in vitro differentiation, enrichment, and transplantation of neural precursor cells from human ES cells. Upon aggregation to embryoid bodies, differentiating ES cells formed large numbers of neural tube-like structures in the presence of fibroblast growth factor 2 (FGF-2). Neural precursors within these formations were isolated by selective enzymatic digestion and further purified on the basis of differential adhesion. Following withdrawal of FGF-2, they differentiated into neurons, astrocytes, and oligodendrocytes. After transplantation into the neonatal mouse brain, human ES cell-derived neural precursors were incorporated into a variety of brain regions, where they differentiated into both neurons and astrocytes. No teratoma formation was observed in the transplant recipients. These results depict human ES cells as a source of transplantable neural precursors for possible nervous system repair.

Nat Biotechnol 2001 Dec;19(12):1129-33

Induced neuronal differentiation of human embryonic stem cells.

Human embryonic stem (ES) cells are pluripotent cells capable of forming differentiated embryoid bodies (EBs) in culture. We examined the ability of growth factors under controlled conditions to increase the number of human ES cell-derived neurons. Retinoic acid (RA) and nerve growth factor (betaNGF) were found to be potent enhancers of neuronal differentiation, eliciting extensive outgrowth of processes and the expression of neuron-specific molecules. Our findings show that human ES cells have great potential to become an unlimited cell source for neurons in culture. These cells may then be used in transplantation therapies for neural pathologies.

Brain Res 2001 Sep 21;913(2):201-5

Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle.

Nuclear reprogramming requires the removal of epigenetic modifications imposed on the chromatin during cellular differentiation and division. The mammalian oocyte can reverse these alterations to a state of totipotency, allowing the production of viable cloned offspring from somatic cell nuclei. To determine whether nuclear reprogramming is complete in cloned animals, we assessed the telomerase activity and telomere length status in cloned embryos, fetuses and newborn offspring derived from somatic cell nuclear transfer. In this report, we show that telomerase activity was significantly ( $P < 0.05$ ) diminished in bovine fibroblast donor cells compared with embryonic stem-like cells, and surprisingly was 16-fold higher in fetal fibroblasts compared with adult fibroblasts ( $P < 0.05$ ). Cell passaging and culture periods under serum starvation conditions significantly decreased telomerase activity by approximately 30-50% compared with nontreated early passage cells ( $P < 0.05$ ). Telomere shortening was observed during in vitro culture of bovine fetal fibroblasts and in very late passages of embryonic stem-like cells. Reprogramming of telomerase activity was apparent by the blastocyst stage of postcloning embryonic development, and telomere lengths were longer (15-23 kb) in cloned fetuses and offspring than the relatively short mean terminal restriction fragment lengths (14-18 kb) observed in adult donor cells. Overall, telomere lengths of cloned fetuses and newborn calves (approximately 20 kb) were not significantly different from those of age-matched control animals ( $P > 0.05$ ). These results demonstrate that cloned embryos inherit genomic modifications acquired during the donor nuclei's in vivo and in vitro period but are subsequently reversed during development of the cloned animal.

Proc Natl Acad Sci U S A 2001 Jan 30;98(3):1077-82

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Normal telomere lengths found in cloned cattle.

Success of cloning using adult somatic cells has been reported in sheep, mice and cattle. The report that "Dolly" the sheep, the first clone from an adult mammal, inherited shortened telomeres from her cell donor and that her telomeres were further shortened by the brief culture of donor cells has raised serious scientific and public concerns about the 'genetic age' and potential developmental problems of cloned animals. This observation was challenged by a recent report that showed calves cloned from fetal cells have longer telomeres than their age-matched controls. The question remains whether Dolly's short telomeres were an exception or a general fact, which would differ from the telomeres of fetal-derived clones.

Nat Genet 2000 Nov;26(3):272-3

Extension of cell life span and telomere length in animals cloned from senescent somatic cells.

The potential of cloning depends in part on whether the procedure can reverse cellular aging and restore somatic cells to a phenotypically youthful state. Here, we report the birth of six healthy cloned calves derived from populations of senescent donor somatic cells. Nuclear transfer extended the replicative life span of senescent cells (zero to four population doublings remaining) to greater than 90 population doublings. Early population doubling level complementary DNA-1 (EPC-1, an age-dependent gene) expression in cells from the cloned animals was 3.5- to 5-fold higher than that in cells from age-matched (5 to 10 months old) controls. Southern blot and flow cytometric analyses indicated that the telomeres were also extended beyond those of newborn (<2 weeks old) and age-matched control animals. The ability to regenerate animals and cells may have important implications for medicine and the study of mammalian aging.

Science 2000 Apr 28;288(5466):665-9

Stem cells: progress in research and edging towards the clinical setting.

Mouse embryonic stem cells have been shown to differentiate into a variety of tissues in vitro and in transplantation experiments can produce many different cell types. Multipotent stem cells in adult humans have also shown a high degree of plasticity: haemopoietic stem cells, for example, have been shown to contribute to several other tissues, such as liver. From these simple observations there has been considerable extrapolation into the use of such putative totipotent stem cells in the clinical setting, with the development of 'designer' tissue engineering, whose aim is to create large tissues or even whole organs for clinical use. In practical terms, however, there are many limitations and difficulties and clinical use has been restricted to a very few settings, eg the use of fetal cells in Parkinson's disease. Nonetheless, there is enormous potential in this area, and also in the application of embryonic or adult stem cells as carriers for gene therapy; but the limitations of such treatment, in particular the stability of manipulated cells, and the problems of aging and Oncogenicity, not to mention a host of ethical and regulatory issues, all need to be considered.

Clin Med 2001 Sep-Oct;1(5):378-82

Medical perspectives on cloning, preimplantation genetic diagnosis, therapeutic use of pluripotent stem cells and the availability of molecular information on the genome.

The progress in biological techniques of cloning, preimplantation genetic diagnosis (PGD) and stem cell production and application of genomic information being generated on human diseases and traits will profoundly influence our lives and progeny produced during the next century. These procedures are controversial with a restricted social acceptance. Cloning by splitting of the fertilized preimplantation stage embryo or by nuclear transplantation procedures will benefit both the agriculture and biotechnology industries. Although monozygotic twins are natural human clones presumably derived from splitting of the single ovum, benefits of human cloning remain unclear. This procedure may not be acceptable near term to a large population. The PGD is a safe procedure for prevention of genetic diseases due to chromosomal anomalies and gene mutations. It can be performed before transfer of the embryo to the recipient natural or foster mother avoiding the trauma of induced abortion when the embryo is found to be abnormal. This technique also improves human fertility caused by anomalous embryo as they may be sorted out by genetic screening before transfer into the maternal uterus. Finally, the therapeutic application of stem cells derived from embryonic cells is wide and novel. They are to be used for therapy of defective organs. Availability of in-depth genomic information will permit characterization of cells for use in all these procedures. Thus, a cautious application of these biological procedures, and the use of

genetic information in the future medical practice will undoubtedly help eradication of human diseases and enhance the quality of our lives.

Early Pregnancy 2001 Jan;5(1 Pt 1):16-17

Bioethics: cloning announcement sparks debate and scientific skepticism.

A small U.S. biotech firm made headlines around the world last week when it announced that it had cloned several human embryos for transplantation research, prompting strong reactions. President George W. Bush denounced the research as unethical, European leaders discussed national controls on cloning, and the furor could spur efforts to pass a law in the United States that would ban research on human cloning. All this fuss over results whose scientific significance is questionable: Some scientists note that the six-cell clusters created by ACT barely qualify as embryos.

Science 2001 Nov 30;294(5548):1802-3

Human cloning from the perspective of The Council of Europe bioethical standards

Allegations negating the role of law in the resolving the controversial problem of human cloning are unjustified. Human rights, implying the magnitude and value of human person and deeply rooted inherent dignity of the human being, constitute the very foundation of every legal order. Every lawyer, as well as every specialist in medicine or biology must be aware of his own human dignity and the ethical consequences. Among the standards of the Council of Europe in the context of human cloning there must be mentioned the Additional Protocol of January 12, 1998 on the Prohibition of Cloning Human Beings. It is the integral element of the normative system of the mother convention, the European Bioethical Convention of April 4, 1997. It has the distinguished place - within this system as far as its substantial provisions exclude the possibilities of limitations and derogation. The system of the Convention and the Protocol must be viewed in the light of a broader normative environment, including the integral system of the European Convention on Human Rights and the set of recommended bioethical standards. The absolute prohibition embodied in the Protocol is limited to all the methods leading to the creation of genetically identical human beings. The protocol does not directly regulate cloning of human tissues and cells, including embryonic stem cells. However, some conclusions may be taken from the Convention and from the recommended standards. It is the assumption of the Convention and the Protocol that the guarantees embodied there must be apprehended as practical and effective ones, justifiable and not excluding the use of proper sanctions by the State. It is an unacceptable view that scientists are excluded from the sphere of the functioning of the above-mentioned prohibition. The real sense of this prohibition is to stop the unlimited liberty and arbitrary practice of such scientists. The freedom of scientific research is not absolute, and must be guided by the respect for human dignity and human rights, as by well as protective guarantees embodied in the Convention and recommended standards.

Med Wieku Rozwoj 2001;V(1 Suppl 1):213-225

Human cloning in the activities of the European Union.

The European Union has been concerned with human cloning since the late 80s. It resulted from inclusion of biotechnology into the sphere of European integration. The attitude of the European Union in the domain of human cloning was shaped, in principle in the second part of the 90s. As the Community law stands at present, the European Union is not able to regulate all aspects of the cloning of human beings. It has no general power to decide in that sphere, especially, as far as bioethic aspects are concerned. The cloning of human beings in the European Union is understood as a process aiming at producing new human being, genetically identical with another live or dead human being. Thus the notion of human cloning is reduced to reproductive cloning. Three instruments are at the disposal of the European Union in the domain of human cloning. The first is prohibition of reproductive cloning as a general principle of Community law. However, that principle is not the result of judicial activity of the European Court of Justice (as general principles normally are), but the logical consequence of views formally expressed by the European Parliament, the Council of the Europe as well as the Commission. The principle was finally included in the Charter of fundamental rights of the European Union. The second instrument is an imperative prohibition of patent granting to biotechnological inventions on human reproductive cloning. Last, but not least, the Union applies a prohibition of financing scientific research connected with human cloning from the budget of the European Communities within the V Framework Programme in the field of research and technological development.

Med Wieku Rozwoj 2001;V(1 Suppl 1):195-212

Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle.

Nuclear reprogramming requires the removal of epigenetic modifications imposed on the chromatin during cellular differentiation and division. The mammalian oocyte can reverse these alterations to a state of totipotency, allowing the production of viable cloned offspring from somatic cell nuclei. To determine whether nuclear reprogramming is complete in cloned animals, we assessed the telomerase activity and telomere length status in cloned embryos, fetuses and newborn offspring derived from somatic cell nuclear

transfer. In this report, we show that telomerase activity was significantly ( $P < 0.05$ ) diminished in bovine fibroblast donor cells compared with embryonic stem-like cells, and surprisingly was 16-fold higher in fetal fibroblasts compared with adult fibroblasts ( $P < 0.05$ ). Cell passaging and culture periods under serum starvation conditions significantly decreased telomerase activity by approximately 30 to 50% compared with nontreated early passage cells ( $P < 0.05$ ). Telomere shortening was observed during in vitro culture of bovine fetal fibroblasts and in very late passages of embryonic stem-like cells. Reprogramming of telomerase activity was apparent by the blastocyst stage of postcloning embryonic development, and telomere lengths were longer (15-23 kb) in cloned fetuses and offspring than the relatively short mean terminal restriction fragment lengths (14-18 kb) observed in adult donor cells. Overall, telomere lengths of cloned fetuses and newborn calves (approximately 20 kb) were not significantly different from those of age-matched control animals ( $P > 0.05$ ). These results demonstrate that cloned embryos inherit genomic modifications acquired during the donor nuclei's in vivo and in vitro period but are subsequently reversed during development of the cloned animal.

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