

LE Magazine July 2002

## ABSTRACTS

## Curcumin

The inhibition of the estrogenic effects of pesticides and environmental chemicals by curcumin and isoflavonoids.

Many environmental chemicals and pesticides have been found to be estrogenic and have been shown to stimulate the growth of estrogen receptor-positive (ER-positive) human breast cancer cells. Since it is difficult to avoid human exposure to environmental estrogens, a potentially important area of research is the development of dietary strategies to prevent the stimulated growth of breast tumors by environmental estrogens. In this context, the inhibitory action of curcumin and a combination of curcumin and isoflavonoids were studied in ER-positive human breast cancer cells (MCF-7 and T47D) and ER-negative MDA-MB-231 cells induced by the pesticide o,p'-DDT and the environmental pollutants 4-nonylphenol and 4-octylphenol. The median inhibitory concentration (IC50) for curcumin in T47D cells was 10 microM when measured at either a 48-hr or a 6-day incubation time. The IC50 value for curcumin was within the 8-10 microM range for inhibiting the growth of T47D cells induced by a 10 microM concentration each of 4-nonylphenol, 4-octylphenol, and o, p'-DDT. The IC50 for curcumin in MCF-7 cells induced by 10 microM of either o,p'-DDT, 4-octylphenol, or 4-nonylphenol were 9, 39, and >50 microM, respectively. A combination of curcumin and isoflavonoids was able to inhibit the induced growth of ER-positive cells up to 95%. For MDA-MB-231 cells, the IC50 for curcumin was 17 microM, which was reduced to 11 microM in the presence of 25 microM genistein. Curcumin and genistein induce drastic changes in the morphological shape of both ER-positive and ER-negative cells. Data presented here indicate that a mixture of curcumin and isoflavonoids is the most potent inhibitor against the growth of human breast tumor cells. These data suggest that combinations of natural plant compounds may have preventive and therapeutic applications against the growth of breast tumors induced by environmental estrogens.

Environ Health Perspect 1998 Dec;106(12):807-12

Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells.

We examined the interaction of curcumin, a dietary constituent and chemopreventive compound, with the carcinogen activation pathway mediated by the aryl hydrocarbon receptor (AhR) in MCF-7 mammary epithelial carcinoma cells. Curcumin caused a rapid accumulation of cytochrome P450 1A1 (CYP1A1) mRNA in a time- and concentration-dependent manner, and CYP1A1 monooxygenase activity increased as measured by ethoxyresorufin-O-deethylation. Curcumin activated the DNA-binding capacity of the AhR for the xenobiotic responsive element of CYP1A1 as measured by the electrophoretic-mobility shift assay (EMSA). Curcumin was able to compete with the prototypical AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin for binding to the AhR in isolated MCF-7 cytosol, indicating that it interacts directly with the receptor. Although curcumin could activate the AhR on its own, it partially inhibited the activation of AhR, as measured by EMSA, and partially decreased the accumulation of CYP1A1 mRNA caused by the mammary carcinogen dimethylbenzanthracene (DMBA). Curcumin competitively inhibited CYP1A1 activity in DMBA-treated cells and in microsomes isolated from DMBA-treated cells. Curcumin also inhibited the metabolic activation of DMBA, as measured by the formation of DMBA-DNA adducts, and decreased DMBA-induced cytotoxicity. These results suggest that the chemopreventive effect of curcumin may be due, in part, to its ability to compete with aryl hydrocarbons for both the AhR and CYP1A1. Curcumin may thus be a natural ligand and substrate of the AhR pathway.

Biochem Pharmacol 1998 Jul 15;56(2):197-206

## Theanine

Enhancing effects of green tea components on the antitumor activity of adriamycin against M5076 ovarian sarcoma.

We have investigated the combined treatment of components of green tea with adriamycin against M5076 ovarian sarcoma, which exhibits low sensitivity to adriamycin. In M5076 tumor-bearing mice, the injection of adriamycin alone did not inhibit tumor growth, whereas the combination of theanine and adriamycin significantly reduced the tumor weight to 62% of the control level. When combined with theanine, effective antitumor activity of adriamycin was observed without an increase in the dosage. Theanine specifically increased the adriamycin concentration in the tumor by 2.7-fold. In contrast, theanine decreased the adriamycin concentrations in normal tissues. On the other hand, in vitro experiments proved that theanine inhibited the efflux of adriamycin from tumor cells, suggesting a theanine-induced increase in the adriamycin concentration in such tumors in vivo. Furthermore, the oral

administration of theanine or green tea similarly enhanced the antitumor activity of adriamycin. In conclusion, the combination of theanine with adriamycin showed antitumor efficacy in spite of the non-effective dose of adriamycin on M5076 ovarian sarcoma. We have found that the modulating action of theanine is useful in clinical cancer chemotherapy.

Cancer Lett 1998 Nov 13;133(1):19-26

Combination of theanine with doxorubicin inhibits hepatic metastasis of M5076 ovarian sarcoma.

Theanine is a peculiar amino acid existing in green tea leaves, which was previously indicated to enhance the antitumor activity of doxorubicin. In the present study, the effect of combination of theanine with doxorubicin against hepatic metastasis of M5076 ovarian sarcoma was investigated. The primary tumor was significantly reduced by the combined treatment on M5076 transplanted (s.c.) mice. The liver weight of control mice increased to twice the normal level because of hepatic metastasis of M5076. In contrast, the injection of doxorubicin alone or theanine plus doxorubicin suppressed the increase in liver weight and inhibited hepatic metastasis. Moreover, the liver weights and metastasis scores demonstrated that theanine enhanced the inhibition of hepatic metastasis induced by doxorubicin. Furthermore, in vitro experiments indicated that theanine increased the intracellular concentration of doxorubicin remaining in M5076 cells. This action suggests that theanine leads the enhancement of the suppressive efficacy of doxorubicin on hepatic metastasis in vivo. Therefore, it was proved that theanine increased not only the antitumor activity on primary tumor but also the metastasis-suppressive efficacy of doxorubicin. The effect of theanine on the efficacy of antitumor agents is expected to be applicable in clinical cancer chemotherapy.

Clin Cancer Res 1999 Feb;5(2):413-6

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Efficacies of tea components on doxorubicin induced antitumor activity and reversal of multidrug resistance.

Considering the novel biochemical modulation by some foods and beverages, we have performed screening for green tea components that have enhancing effects on doxorubicin (DOX) induced antitumor activity. Components, such as caffeine, theanine, epigallocatechin gallate (EGCG) and flavonoids have inhibitory effects on the DOX efflux from Ehrlich ascites carcinoma cells. Thus, it is suggested that EGCG and flavonoids may enhance DOX induced antitumor activity and increase the DOX concentrations in tumors through the inhibition of DOX efflux. It is expected that these components in green tea exhibit low toxicity and that there are few side effects of drinking green tea in combination with an antitumor agent. We think that the intake of a favorite beverage favors a positive mental attitude of a patient and increases the efficacy of the chemotherapeutic index, and that this efficacy is useful for improving the quality of life while on cancer chemotherapy. In DOX resistant P388 leukemia cell bearing mice theanine increased the DOX induced efficacy through an increase in the DOX concentrations in the tumors. Theanine attacked the same transport process for DOX in both types of cells, elevated the DOX concentration and increased the DOX induced antitumor activity.

Toxicol Lett 2000 Apr 3;114(1-3):155-62

Enhancement of the activity of doxorubicin by inhibition of glutamate transporter.

Theanine enhanced doxorubicin (DOX) induced antitumor activity by increasing the concentration of DOX in the tumor through the inhibition of efflux of DOX from tumor cells. As theanine reduced the level of glutamate via suppression of the glutamate transporter in tumor cells, we studied the change in the intracellular concentration of glutathione (GSH) and the correlation with the GSH S-conjugate export (GS-X) pump. The reduction in the concentration of glutamate in tumor cells caused by theanine, induced decreases in the intracellular GSH and GS-DOX levels. The expression of MRP5 in M5076 cells, was confirmed. We concluded that the GS-DOX conjugate was transported extracellularly via the MRP5/GS-X pump in M5076 cells and that theanine affected this route. Namely, theanine increases the concentration of DOX in a tumor in vivo through inhibition of the glutamate transporter via the GS-X pump.

Toxicol Lett 2001 Sep 15;123(2-3):159-67

Improvement of idarubicin induced antitumor activity and bone marrow suppression by theanine, a component of tea.

We have examined the effect of theanine, a specific amino acid in green tea, on idarubicin (IDA)-induced antitumor activity and toxicity. In combination with theanine, IDA (0.25 mg/kg per day x 4 days, a dose that does not show antitumor activity) had significant antitumor activity in P388-bearing mice. The IDA concentration in the tumors in the theanine plus IDA group increased to twice the level in the IDA alone group. Furthermore, the decrease in tumor weight caused by IDA at 1.0 mg/kg per day x 4 days (at this dose IDA exhibits antitumor activity) was significantly amplified by theanine. The numbers of leukocyte and bone marrow cells decreased significantly on IDA injection. Theanine significantly reversed these changes. These results suggest that theanine selectively moderates the IDA-induced toxicities. Until recently, the antitumor activity and related toxicities of this chemotherapeutic agent in leukemia could not be distinguished. Theanine increases the IDA-induced antitumor activity and ameliorates the toxicities.

Cancer Lett 2000 Oct 1;158(2):119-24

Arginine

Effect of fish oil, arginine, and doxorubicin chemotherapy on remission and survival time for dogs with lymphoma: a double-blind, randomized placebo-controlled study.

**BACKGROUND:** Polyunsaturated n-3 fatty acids have been shown to inhibit the growth and metastasis of tumors. This double-blind, randomized study was designed to evaluate the hypothesis that polyunsaturated n-3 fatty acids can improve metabolic parameters, decrease chemical indices of inflammation, enhance quality of life, and extend disease free interval and survival time for dogs treated for lymphoblastic lymphoma with doxorubicin chemotherapy. **METHODS:** Thirty-two dogs with lymphoma were randomized to receive one of two diets supplemented with menhaden fish oil and arginine (experimental diet) or an otherwise identical diet supplemented with soybean oil (control diet). Diets were fed before and after remission was attained with up to five dosages of doxorubicin. Parameters examined included blood concentrations of glucose, lactic acid, and insulin in response to glucose and diet tolerance tests; alpha-1 acid glycoprotein; tumor necrosis factor; interleukin-6; body weight; amino acid profiles; resting energy expenditure; disease free interval (DFI); survival time (ST); and clinical performance scores. **RESULTS:** Dogs fed the experimental diet had significantly ( $P < 0.05$ ) higher mean serum levels of the n-3 fatty acids docosahexaenoic acid (C22:6) and

eicosapentaenoic acid (C20:5) compared with controls. Higher serum levels of C22:6 and C20:5 were associated with lesser ( $P < 0.05$ ) plasma lactic acid responses to intravenous glucose and diet tolerance testing. Increasing C22:6 levels were significantly ( $P < 0.05$ ) associated with longer DFI and ST for dogs with Stage III lymphoma fed the experimental diet. CONCLUSIONS: Fatty acids of the n-3 series normalize elevated blood lactic acid in a dose-dependent manner, resulting in an increase in DFI and ST for dogs with lymphoma.

Cancer 2000 Apr 15;88(8):1916-28

Arginase activity in human breast cancer cell lines: N(omega)-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells.

L-Arginine is the common substrate for two enzymes, arginase and nitric oxide synthase (NOS). Arginase converts L-arginine to L-ornithine, which is the precursor of polyamines, which are essential components of cell proliferation. NOS converts L-arginine to produce NO, which inhibits proliferation of many cell lines. Various human breast cancer cell lines were initially screened for the presence of arginase and NOS. Two cell lines, BT-474 and MDA-MB-468, were found to have relatively high arginase activity and very low NOS activity. Another cell line, ZR-75-30, had the highest NOS activity and comparatively low arginase activity. The basal proliferation rates of MDA-MB-468 and BT-474 were found to be higher than the ZR-75-30 cell line. N-Hydroxy-L-arginine (NOHA), a stable intermediate product formed during conversion of L-arginine to NO, inhibited proliferation of the high arginase-expressing MDA-MB-468 cells and induced apoptosis after 48 h. NOHA arrested these cells in the S phase, increased the expression of p21, and reduced spermine content. These effects of NOHA were not observed in the ZR-75-30 cell line, which expresses high NOS and relatively low arginase. The effects of NOHA were antagonized in the presence of L-ornithine (500 microM), which suggests that in MDA-MB-468 cell line, the arginase pathway is very important for cell proliferation. Inhibition of the arginase pathway led to depletion of intracellular spermine and apoptosis as observed by terminal deoxynucleotidyl transferase (TdT)-mediated nick end labeling assay and induction of caspase 3. In contrast, the ZR-75-30 cell line maintained its viability and its L-ornithine and spermine levels in the presence of NOHA. We conclude that NOHA has antiproliferative and apoptotic actions on arginase-expressing human breast cancer cells that are independent of NO.

Cancer Res 2000 Jun 15;60(12):3305-12

Inhibition of the growth of human pancreatic cancer cells by the arginine antimetabolite L-canavanine.

L-Canavanine (CAV), the L-2-amino-4-guanidinoxy structural analogue of L-arginine (ARG), is a potent ARG antagonist which occurs in the jack bean, *Canavalia ensiformis*. This ARG antimetabolite is active against L1210 murine leukemia and a solid colonic tumor in the rat. Our initial studies using a microtiter assay show that CAV exhibits a 50% inhibitory concentration of approximately 2 mM against the human pancreatic adenocarcinoma cell line, MIA PaCa-2, when these cells are grown in Dulbecco's modified Eagle's medium containing 0.4 mM ARG. When the ARG concentration is reduced to 0.4 microM, the 50% inhibitory concentration for CAV falls precipitously to 0.01 mM. The pronounced increase in the ability of CAV to inhibit MIA PaCa-2 cell growth at the lower ARG concentration may result from enhanced CAV competition with ARG for incorporation into newly synthesized cellular proteins. At 0.4 microM ARG, 30 mM CAV almost completely inhibits cell growth by 6 h. In contrast, with 0.4 mM ARG, complete inhibition does not occur until after 48 h. A dramatic reversal of growth inhibition caused by a very high concentration of CAV was observed when cells treated with CAV were replenished with a high concentration of ARG. Our results suggest that CAV has real potential as a lead compound for the development of analogues with enhanced activity against human pancreatic cancer.

Cancer Res 1994 Dec 1;54(23):6045-8

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### Cimetidine

Cimetidine increases survival of colorectal cancer patients with high levels of sialyl Lewis-X and sialyl Lewis-A epitope expression on tumour cells.

Cimetidine has been shown to have beneficial effects in colorectal cancer patients. In this study, a total of 64 colorectal cancer patients who received curative operation were examined for the effects of cimetidine treatment on survival and recurrence. The cimetidine group was given 800 mg day<sup>-1</sup> of cimetidine orally together with 200 mg day<sup>-1</sup> of 5-fluorouracil, while the control group received 5-fluorouracil alone. The treatment was initiated 2 weeks after the operation and terminated after 1 year. Robust beneficial effects of cimetidine were noted: the 10-year survival rate of the cimetidine group was 84.6% whereas that of control group was 49.8% ( $P < 0.0001$ ). According to our previous observations that cimetidine blocked the expression of E-selectin on vascular endothelium and inhibited the adhesion of cancer cells to the endothelium, we have further stratified the patients according to the expression levels of sialyl Lewis antigens X (sL(x)) and A (sL(a)). We found that cimetidine treatment was particularly effective in patients whose tumour had higher sL(x) and sL(a) antigen levels. For example, the 10-year cumulative survival rate of the cimetidine group with higher CSLEX staining, recognizing sL(x) of tumours, was 95.5%, whereas that of the control group was 35.1% ( $P=0.0001$ ). In contrast, in the group of patients with no or low levels CSLEX staining, cimetidine did not show significant beneficial effect (the 10-year survival rate of the cimetidine group was 70.0% and that of the control group was 85.7% ( $P=n.s.$ )). These results clearly indicate that cimetidine treatment dramatically improved survival in colorectal cancer patients with tumour cells expressing high levels of sL(x) and sL(a).

Br J Cancer 2002 Jan 21;86(2):161-7

Clinical improvement in advanced cancer disease after treatment combining histamine and H<sub>2</sub>-antihistaminics (ranitidine or cimetidine).

In a randomized study 31 patients with advanced cancer disease in whom classical anticancer therapy had been abandoned received a daily combination of subcutaneous histamine and oral H<sub>2</sub>-antihistaminics. In 27 patients, treatment induced a marked clinical improvement as shown by a large rise in performance status (Karnofsky scale). Ten patients were still alive 3-14 months after initiation of treatment. Average survival in the 31 treated patients (172 +/- 113 days) was significantly longer than in 34 non-treated patients with similar advanced cancer (26 +/- 16 days,  $P$  less than 0.00001). In six treated patients, the size of liver and lung metastases decreased. Histamine was perfectly tolerated up to 4 mg/day.

Eur J Cancer Clin Oncol 1988 Feb;24(2):161-7

Effect of cimetidine on survival after gastric cancer.

The effect of cimetidine on survival was investigated in 181 patients with gastric cancer. Immediately after operation or the decision not to operate, the patients were randomized in double-blind fashion to placebo or cimetidine 400 mg twice daily for two years or until death, with review every three months. Median survival in the cimetidine group was 450 days (range 1-1826) and in the placebo group 316 days (1-1653). The relative survival rates (cimetidine/placebo) were 45%/28% at 1 year, 22%/13% at 2 years, 13%/7% at 3 years, 9%/3% at 4 years, and 2%/0% at 5 years. Survival in the cimetidine group was significantly longer than in the placebo group.

Lancet 1988 Oct 29;2(8618):990-2

Prevention of alterations in postoperative lymphocyte subpopulations by cimetidine and ibuprofen.

Surgical procedures probably result in a temporary state of immunosuppression. Identification of functional lymphocyte subclasses using appropriate monoclonal antibodies appears to serve as a sensitive, accurate, and reproducible measure of immune status in patients in many disease states. Using monoclonal antibodies specific for lymphocyte surface markers and immunofluorescent assay, we quantitated lymphocyte subpopulations in patients undergoing surgical procedures. Cholecystectomy, colon surgery, and coronary bypass procedures all resulted in postoperative decreases in helper and inducer populations and increases in cytotoxic suppressor populations, with resultant depressions in the helper to suppressor lymphocyte ratio. Studies in an additional group of patients who underwent cholecystectomy demonstrated that these changes could be prevented by perioperative administration of ibuprofen and cimetidine. These results suggest that prostaglandins and histamines are involved in immunoregulatory events after major operation. The ability of specific pharmacologic therapy to prevent alterations in lymphocyte

populations suggest that postoperative immunity may be preserved, hopefully leading to greater host resistance against infection and tumor dissemination.

Am J Surg 1986 Feb;151(2):249-55

Cimetidine preserves non-specific immune function after colonic resection for cancer.

Fifty consecutive patients undergoing resection of colorectal cancer were randomized to either receive cimetidine at a dose of 400 mg bd for a minimum of 5 pre-operative days, then intravenously for 2 postoperative days, or to act as controls. Baseline immune function was determined in all patients by in vitro testing of lymphocyte proliferation (LP) in response to mitogen, skin testing for cell mediated immunity (CMI) and measurement of lymphocyte subsets. Immune function was retested in both groups on the second postoperative day. In control patients the mean postoperative LP value was 41% of pre-operative levels ( $P < 0.0001$ ) and the mean CMI reduced to 29% ( $P < 0.0001$ ). Patients treated with cimetidine had no significant fall in these parameters. Numbers of T and natural killer (NK) cells fell after surgery in both groups, and B cell numbers were maintained in the cimetidine group. It is concluded that cimetidine reduces the immunosuppression that follows colonic resection.

Aust N Z J Surg 1994 Dec;64(12):847-52

The growth of carcinogen-induced colon cancer in rats is inhibited by cimetidine.

Colon cancer was induced in 40 Sprague Dawley rats using a 10-week course of 1,2 dimethylhydrazine (DMH). Twenty animals received cimetidine in their drinking water, commencing 5 weeks after concluding the course of DMH. After five weeks treatment of the animals were sacrificed and the colon and rectum excised. Tumours were assessed histologically for depth of invasion, inflammatory cell response and stained for Proliferating Cell Nuclear Antigen (PCNA), as a measure of tumour proliferative index. PCNA staining was measured using a computerized image analysis system. There were 25 tumours in the cimetidine treated group and 20 in controls. In the control group, 10% of the tumours were benign, 35% malignant polyps, 40% invading through submucosa and 15% invading through the bowel wall, as opposed to 40%, 44%, 8% and 8%, respectively in the cimetidine group (Chi squared test:  $P = 0.002$ ). The mean proliferative index for control tumours was 27.9% and for the cimetidine tumours 23.1% t test:  $P = 0.002$ ). It is concluded that cimetidine inhibits colon cancer cellular proliferation and slows early tumour invasion in this animal model.

Eur J Surg Oncol 1993 Aug;19(4):332-5

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### Regenerative Medicine

Cytokines and neurotrophic factors fail to affect Nogo-A mRNA expression in differentiated human neurones: implications for inflammation-related axonal regeneration in the central nervous system.

Nogo is a novel myelin-associated inhibitor of neurite outgrowth which regulates stable neuronal connections during axonal regeneration following injury in the adult mammalian central nervous system (CNS). Because cytokines and neurotrophic factors play a key role in inflammation-related axonal regeneration, we investigated: (i) the constitutive expression of Nogo and the Nogo receptor (NgR) mRNA in human neural cell lines; (ii) Nogo and NgR mRNA levels in the NTera2 human teratocarcinoma cell line during retinoic acid (RA)-induced neuronal differentiation; and (iii) their regulation in NTera2-derived differentiated neurones (NTera2-N) after exposure to a battery of cytokines and growth factors potentially produced by activated glial cells at post-traumatic inflammatory lesions in the CNS. By reverse transcriptase-polymerase chain reaction analysis, the constitutive expression of Nogo-A, the longest isoform of three distinct Nogo transcripts and NgR mRNA was identified in a wide variety of human neural and non-neural cell lines. By Northern blot analysis, the levels of Nogo-A mRNA were elevated markedly in NTera2 cells following RA-induced neuronal differentiation, accompanied by an increased expression of the neurite growth-associated protein GAP-43 mRNA. In contrast, Nogo-A, Nogo-B, NgR and GAP-43 mRNA levels were unaltered in NTera2-N cells by exposure to basic fibroblast growth factor, brain-derived neurotrophic factor, glia-derived neurotrophic factor, tumour necrosis factor-alpha, interleukin-1beta, dibutyryl cyclic AMP or phorbol 12-myristate 13-acetate. These results indicate that both Nogo-A and NgR mRNA are coexpressed in various human cell types, including differentiated neurones, where their expression is unaffected by exposure to a panel of cytokines and neurotrophic factors which might be involved in inflammation-related axonal regeneration in the CNS.

Neuropathol Appl Neurobiol 2002 Apr;28(2):95-106

Messenger-RNA-binding proteins and the messages they carry.

From sites of transcription in the nucleus to the outreaches of the cytoplasm, messenger RNAs are associated with RNA-binding proteins. These proteins influence pre-mRNA processing as well as the transport, localization, translation and stability of mRNAs. Recent discoveries have shown that one group of these proteins marks exon-exon junctions and has a role in mRNA export. These proteins communicate crucial information to the translation machinery for the surveillance of nonsense mutations and for mRNA localization and translation.

Nat Rev Mol Cell Biol 2002 Mar;3(3):195-205

cDNA cloning confirms the polyadenylation of RNA decay intermediates in *Streptomyces coelicolor*.

In *Escherichia coli* the poly(A) tails of messenger and rRNAs are a major determinant of RNA stability. These tails are formed primarily by poly(A) polymerase I (PAP I) in wild-type strains or by polynucleotide phosphorylase (PNPase) in PAP I-deficient strains. In *Streptomyces coelicolor* it has been shown that mycelial RNAs display biochemical characteristics consistent with the presence of poly(A) tails. To confirm the occurrence of polyadenylation, rRNA and mRNA transcripts from *S. coelicolor* were isolated by oligo(dT)-dependent RT-PCR followed by cDNA cloning. One of the clones obtained was polyadenylated at a site corresponding to the mature 3' terminus of 16S rRNA, while two 23S rRNA cDNA clones were polyadenylated at precursor processing sites. Other clones identified polyadenylation sites internal to the coding regions of both 16S and 23S rRNAs, and redD and actII-orf4 mRNAs. While most rRNA cDNA clones displayed adenosine homopolymer tails, the poly(A) tails of three rRNAs and all the redD and actII-orf4 clones consisted of a variety of heteropolymers. These results suggest that the enzyme primarily responsible for polyadenylation in *S. coelicolor* is PNPase rather than a PAP I homologue.

Microbiology 2002 May;148(Pt 5):1421-5

Positional effects of short interfering RNAs targeting the human coagulation trigger Tissue Factor.

Chemically synthesised 21-23 bp double-stranded short interfering RNAs (siRNA) can induce sequence-specific post-transcriptional gene silencing, in a process termed RNA interference (RNAi). In the present study, several siRNAs synthesized against different sites on the same target mRNA (human Tissue Factor) demonstrated striking differences in silencing efficiency. Only a few of the siRNAs resulted in a significant reduction in expression, suggesting that accessible siRNA target sites may be rare in some human mRNAs. Blocking of the 3'-OH with FITC did not reduce the effect on target mRNA. Mutations in the siRNAs relative to target mRNA sequence gradually reduced, but did not abolish mRNA depletion. Inactive siRNAs competed reversibly with active siRNAs in

a sequence-independent manner. Several lines of evidence suggest the existence of a near equilibrium kinetic balance between mRNA production and siRNA-mediated mRNA depletion. The silencing effect was transient, with the level of mRNA recovering fully within 4-5 days, suggesting absence of a propagative system for RNAi in humans. Finally, we observed 3' mRNA cleavage fragments resulting from the action of the most effective siRNAs. The depletion rate-dependent appearance of these fragments argues for the existence of a two-step mRNA degradation mechanism.

Nucleic Acids Res 2002 Apr 15;30(8):1757-66

A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*.

A small RNA, RyhB, was found as part of a genomewide search for novel small RNAs in *Escherichia coli*. The RyhB 90-nt RNA down-regulates a set of iron-storage and iron-using proteins when iron is limiting; it is itself negatively regulated by the ferric uptake repressor protein, Fur (Ferric uptake regulator). RyhB RNA levels are inversely correlated with mRNA levels for the *sdhCDAB* operon, encoding succinate dehydrogenase, as well as five other genes previously shown to be positively regulated by Fur by an unknown mechanism. These include two other genes encoding enzymes in the tricarboxylic acid cycle, *acnA* and *fumA*, two ferritin genes, *ftnA* and *bfr*, and a gene for superoxide dismutase, *sodB*. Fur positive regulation of all these genes is fully reversed in a RyhB mutant. Our results explain the previously observed inability of *fur* mutants to grow on succinate. RyhB requires the RNA-binding protein, Hfq, for activity. Sequences within RyhB are complementary to regions within each of the target genes, suggesting that RyhB acts as an antisense RNA. In *sdhCDAB*, the complementary region is at the end of the first gene of the *sdhCDAB* operon; full-length *sdhCDAB* message disappears and a truncated message, equivalent in size to the region upstream of the complementarity, is detected when RyhB is expressed. RyhB provides a mechanism for the cell to down-regulate iron-storage proteins and nonessential iron-containing proteins when iron is limiting, thus modulating intracellular iron usage to supplement mechanisms for iron uptake directly regulated by Fur.

Proc Natl Acad Sci U S A 2002 Apr 2;99(7):4620-5

Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients.

Immune parameters were studied in 86 haemophilia patients (six with AIDS) and 87 healthy controls. We found lymphocytotoxic alloantibodies in HIV-positive (HIV+) sera, which reacted preferentially with B lymphocytes but also with T lymphocytes, and which reacted more frequently at 4 degrees C than at 37 degrees C. The antibodies were not directed against HIV-induced structures on T lymphocytes and they were reactive with both CD4+ and CD8+ lymphocytes. In addition to cytotoxic alloantibodies, cytotoxic autoantibodies were detected, which coated patient lymphocytes *in vivo*. Increased proportions of *in vivo*-antibody-coated-cells were found in 37 of 86 haemophilia patients. Antibody binding was labile so that the immunoglobulins were partially removed from the lymphocyte surface by washing. The autoreactive antibodies were of IgG and IgM type, fixed complement as demonstrated by increased anti-C3d+ cells in the patients' blood, and reacted with CD4+ as well as CD8+ lymphocytes. There was a statistically significant correlation of increased Ig+ cells with HIV infection, decreased CD4/CD8 ratios, increased serum neopterin levels, and abnormal *in-vitro* responses to pooled allogeneic stimulator cells or CD3 monoclonal antibody. Patients with increased Ig+ cells were lymphopenic, had decreased absolute counts of CD4+, CD25+, CD21+ and OKM5+ cells, and higher percentages of CD8+ and OKIa1+ cells in their blood than patients with normal levels of Ig+ cells. Our data suggest a role of autoreactive anti-lymphocyte antibodies in the pathogenesis of acquired immunodeficiency.

Clin Exp Immunol 1989 Feb;75(2):178-83

Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice.

We present genome-wide microarray expression analysis of 11,000 genes in an aging potentially mitotic tissue, the liver. This organ has a major impact on health and homeostasis during aging. The effects of life- and health-span-extending caloric restriction (CR) on gene expression among young and old mice and between long-term CR (LT-CR) and short-term CR (ST-CR) were examined. This experimental design allowed us to accurately distinguish the effects of aging from those of CR on gene expression. Aging was accompanied by changes in gene expression associated with increased inflammation, cellular stress, and fibrosis, and reduced capacity for apoptosis, xenobiotic metabolism, normal cell-cycling, and DNA replication. LT-CR and just 4 weeks of ST-CR reversed the majority of these changes. LT-CR produced in young mice a pattern of gene expression that is a subset of the changes found in old LT-CR mice. It is possible that the early changes in gene expression, which extend into old age, are key to the life- and health-span-extending effects of CR. Further, ST-CR substantially shifted the "normo-aging" genomic profile of old control mice toward the "slow-aging" profile associated with LT-CR. Therefore, many of the genomic effects of CR are established rapidly. Thus, expression profiling should prove useful in quickly identifying CR-mimetic drugs and treatments.

Proc Natl Acad Sci U S A 2001 Sep 11;98(19):10630-5

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