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Gene Expression Programming to Envelop Metastasized Cancer Cells by Commanding Adjacent Fibroblasts

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Abstract: We have developed the novel Fuzzy Set Chaos Algorithm (FSCA) to regulate tissue histology. If serum quantities of molecules that activate the expression of genes and proteins are at fuzzy levels, then histological regulation could be accomplished by iterative ON/OFF expression programming cycles and the resulting plot-pattern would render a depiction of chaos. We tested this hypothesis on metastasized cancer cells that proliferate rapidly and migrate into adjacent tissues and organs. Using a basic flow chart of histological regulation, we searched various databases to identify effective molecules. These molecules were then used for gene expression programming to envelop metastasized tumor cells by commanding fibroblasts to surround the target cells in the tissue. The ON commands we used were 1) switch on transcription of collagen genes by ascorbic acid (VC), 2) increase collagen mRNA translation by supplying collagen-enriched food or by amino acids Pro Gly and Cys, 3) envelop metastasized cancer cells by combining collagen with elastin which is activated by calcium, and 4) proliferate fibroblastic cells by thiamin (VB1), riboflavin (VB2), pyridoxine (VB6), and cobalamin (VB12) during the fibrogenesis. Elimination of the supply of individual molecules constituted the OFF command. The commands were transmitted via the blood flow; their delivery to the area of interest was possible because permeability of the walls of blood vessels around metastasized cancer cells is increased by IL-1, IL-6, TNF-α, PAF, prostacyclin bradykinin and VEGF. Cytokines and chemokines around metastasized cancer cells served to inform fibroblasts of the location of metastasized cancer cells. Successful of fibrogenesis was conformed by rat liver fibrosis and the effectiveness of the therapy by patient viability.

Keywords: *Malignant Tumor, Metastasis, GEP Therapy, Scirrhous Stomach Cancer Cured*

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Introduction

Historical data indicate that alcohol has been consumed by humans since before 3000 BC. Alcohol is thought to be an inducer molecule of fibrosis. Except for rats receiving high-fat diets and baboons, efforts to induce experimental alcoholic fibrosis in animals receiving regular diets have failed.

In 1996, we succeeded in inducing alcoholic fibrosis in the livers of rats provided with high doses of alcohol (50-70%) in their drinking water (1) without using artificial tubing drinking. In that experiment, the animals received an alternating autoclaved (atCE2) and non-autoclaved standard diet (CE2). The alternating feeding pattern was based on the results of a survey of the eating and drinking habits of alcoholic patients. The atCE2 diet was used as a specific pathogen-free (SPF) diet and the CE2 diet was used as a non-SPF diet. Autoclaving the diet degrades VB1, VC, retinol (VA), and VB12.

To investigate the mechanism(s) of experimental fibrogenesis induction (1), we performed a database analysis and found that ascorbic acid (VC) induces collagen synthesis (2-5) (the ON switch for collagen genes) and VB1 produces ATP energy (6) (the ON switch for cell activation). Furthermore, decreased VA reduces the expression of RARE-carrying genes such as the fibronectin gene whose product plays a role in cell migration (7,8), that is, reduced VA induces the fixation of tissues (organ) to stop tissue (organ) gelation (the OFF switch for cell migration) by preventing the migration of functional cells from the tissue (organ). VB12 induces mitosis of megaloblastic bone marrow-, stromal osteoprogenitor- and osteoblastic cells (9,10), suggesting that decreased VB12 might switch OFF the mitosis of structural cells such as hepatocytes in our liver fibrosis.

Our paper describes the successful application to human disease treatment of gene expression programming that is dispatched to envelope metastasized malignant cells with collagens, elastin and fibroblasts. This programming technique was used in a clinical setting after we succeeded in inducing fibrogenesis in the liver of alcoholic rats.

In the current study we analyzed the main causative molecules that could lead to liver fibrosis in those rats and found at a minimum, vitamin C (VC), calcium and vitamin B1 were required. The concomitant presence of proline, glycine, and cysteine, the specific amino acid components of collagens I, III, & IV, led to the induction of severe fibrosis. We were unable to induce fibrogenesis in our rats with only VC or an excess of vitamin A.

Our major approach is based on a no risk - high return concept. In the beginning we thought that 6 months or more would be required to alter tissue histology, therefore we chose molecules that have low side effects but are imbued with excellent gene and/or protein expression. The molecules we used for gene expression programming presented low risk on the one hand, but offered a high return for health on the other.

We applied our gene expression programming technology to achieve the envelopment of metastasizing malignant human cells. After determining that VC elevated the profilin mRNA levels, we intravenously injected VC-induced profilin gene expression into the liver of rats drinking alcohol.

We suggest that the findings reported in this paper are very important because gene expression programming therapy may save the lives of patients struck with incurable diseases. The application of this therapy may prove invaluable not only in efforts to suppress cancer cell metastasis, but also in the treatment of other currently incurable or recurring diseases (e.g. low back pain, arthritis, liver cirrhosis, lung fibrosis, diabetes mellitus, atopic dermatitis, angina pectoris, arteriosclerosis, osteoporosis). The ability to command fibroblasts or target cells to reconstruct histology represents a

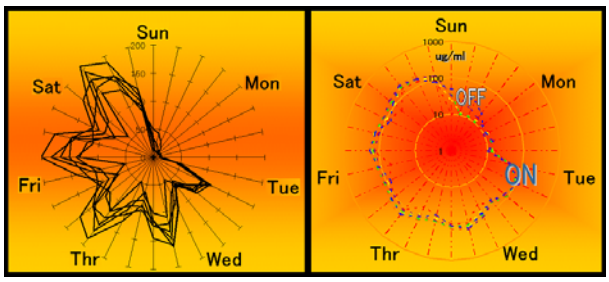


Figure 1. Fuzzy Chaos Algorithm illustrated by weekly alternating diet regimens in which vitamin C (VC) was used to produce ON/OFF gene expression cycles. The serum levels of VC reflected oral VC intake: VC in the diets affected these levels and subsequent cycles became fuzzy. Repeating the alternating feeding regimens at weekly intervals resulted in gene expression cycles that mimicked chaos. The one-day time course in serum VC levels after the administration of VC 1 g/person was analyzed at 10 and 30 min, and at 1, 3, 6, 12, and 24 hr after administration. The body weight of the 5 subjects was 53 ± 7.7 (SD) kg. Prior maximum informed consent was obtained from all participants. Time course analysis of orally administered VC showed maximum serum VC levels at 3 hr; they returned to the ordinal level after 24 hr.

big step forward in the treatment and/or cure of human diseases. We wish to encourage other investigators to study and improve our methodology because it offers a low-risk,

high-return means of treating common and serious human diseases by taking advantage of cutting-edge technology.

In the current study we applied our FSCA to the suppression of metastasized cancer cells. If we were successful in enveloping metastasized cancer cells with fibroblasts by commanding them to surround these cancer cells, produce collagens, and bundle collagen fibers with post-activated elastin using calcium for fixation, we might be able to induce necrosis or death of metastasized malignant cancer cells. We assumed that the induction of necrosis or cell death in metastasized cancer cells is regulated by the high or low dependence on nutrients of metastasized cells and surrounding fibroblasts.

Profilin cDNA was obtained by Fuzzy Set Cloning (FSC)(11, 12); activated profilin gene expression was detected by the defuzzification process in CCl4-induced liver cirrhosis in rats. (13) In the same manner, extracellular matrix (ECM) gene expression levels were analyzed during granulation of flexor tendons (14). The fibronectin and collagen genes were expressed after time lags (11-16). We speculated that for the artificial regulation of histological reconstruction, we could use our fuzzy set ON/OFF switching to program gene expression for each gene set.

Gene expression molecules and information obtained by searching the Medline database

To regulate gene expression, we wanted to identify non-toxic, long-term usable, non-antigenic molecules. We found in the Medline database (Fig. 2) the following vitamins as gene expression inducer molecules: VA activates fibronectin (17), laminin B1 (18), and the bone sialoprotein (19) gene by binding to the retinoic acid responsive element (RARE). VC activates collagen (2-4) and profilin (Fig. 3A & C). One of binding factors of VC is the core binding factor 1 (Cbfa1). (20) VD activates ECM metalloproteinase gene expression by binding VD responsive elements (21). VB1 functions in ATP energy production in the tricarboxylic acid (TCA) cycle, acting as the co-enzyme of oxidative decarboxylation and α-ketoglutarate dehydrogenase that catalyzes transketolase reactions.

Experimentally analyzed molecules for fibrogenesis induced in alcoholic rats

The relationship between fibrosis and vitamins was confirmed in our fibrogenesis induction system in alcoholic rats (1). The initial experiment for gene expression programming was successful: fibrogenesis was induced in alcoholic

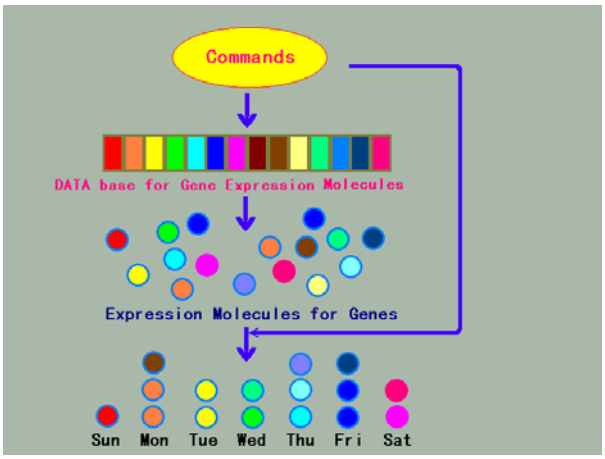


Figure 2. Image flow chart for gene and protein expression programming for weekly switching to reconstruct histological events. Commands are translated into gene or protein expression molecules after database analysis and the histological reconstruction is planned by positioning the molecules on a sequential time architectures to form a one-week cycle after considering the post-expressed character of the protein.

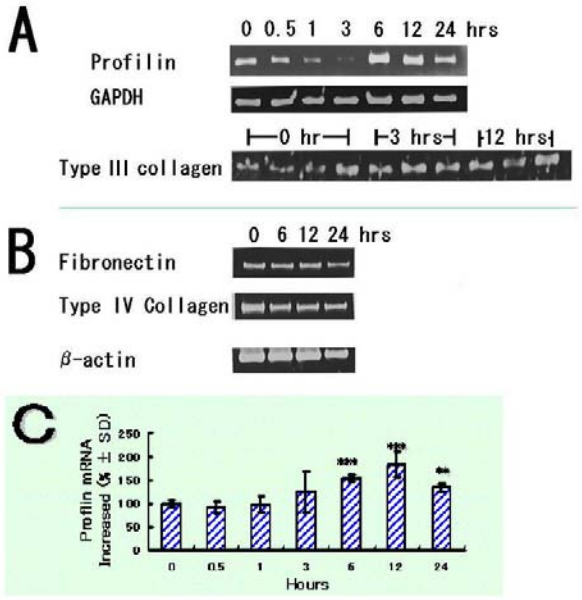
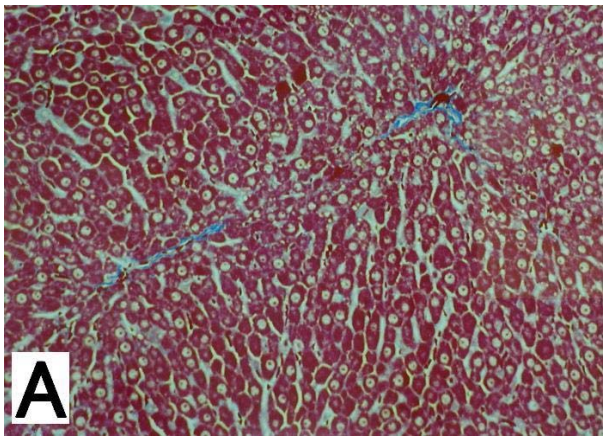
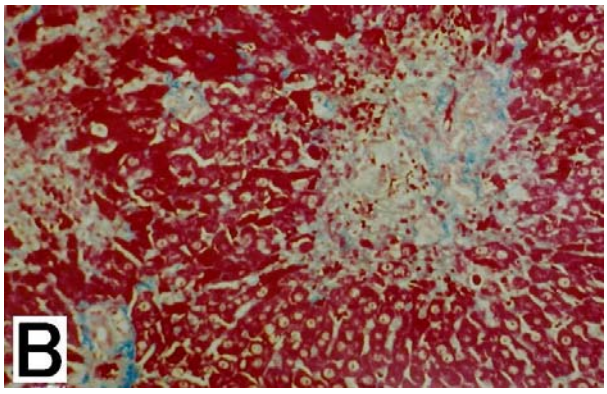
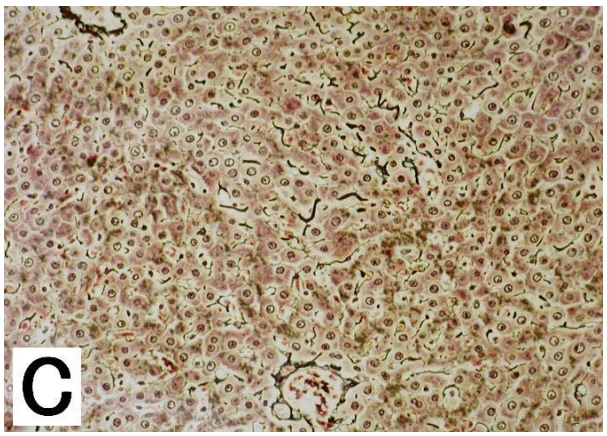


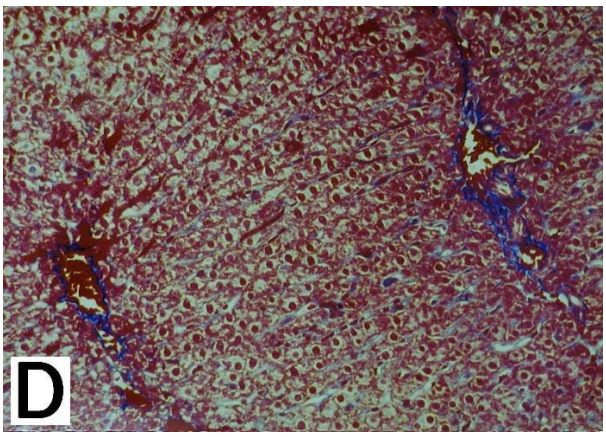
Figure 3. After vitamin C injection, profilin, type III collagen, fibronectin, type IV collagen, GAPDH, and β-actin mRNA levels were analyzed by RT-PCR using samples from alcoholic rats imbibing 70% ethanol that were undergoing liver fibrogenesis. A): Time course analysis of the profilin-, GAPDH-, and type III collagen mRNA levels. B): fibronectin, type IV collagen, and β-actin. C): Profilin mRNA levels after vitamin C injection. Mean ± SD of 3 or 4 rats. Differences between control (0 hr) and 6, 12, 24 hrs were significantly different (***: $p<0.01$, **: $p<0.05$).

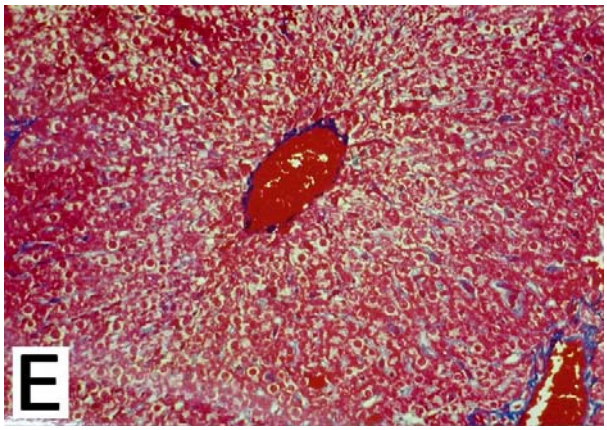
rats (Fig. 4A). In that experiment, we replicated data from a survey of human alcoholics, which indicated that they drank high doses of alcohol and ate dried foods, but rarely ate nutrient enriched foods. These dietary patterns were simulated by feeding the rats alternating weekly diets of autoclaved (atCE2) and regular food (CE2). VC, VB1, retinol (VA), and VB12 were depleted by autoclaving. We inferred that major activation modulators of this gene expression system were increased VC and decreased retinol.











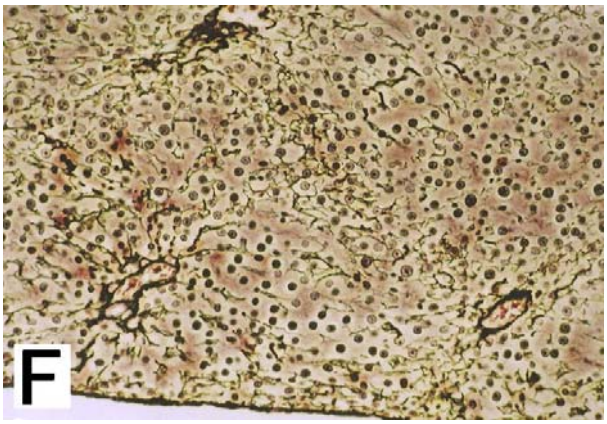


Figure 4 A-F. Liver fibrosis induced in alcoholic rats by gene and protein expression programming. A, B and C indicate fibrosis induced by alternating atCE2 and supplemented diets (VC: 5.7 mmole/50 kgbw/d: 50 kg body weight/day, VB1: 0.57 mmole/50 kgbw/d, and calcium: 5.7 mmole/50 kgbw/d). A: minimal fibrosis, B: periportal fibrosis in middle level steatosis, C: mild pericellular fibrosis. D, E and F are the results from mice fed alternating atCE2 and atCE2 diets supplemented with VC, VB1, Ca, Pro (4.5 mmole/50 kgbw/d), Gly (5.7 mmole/50 kgbw/d), Cys (1.1 mmole/50 kgbw/d). D: periportal fibrosis, E: pericellular fibrosis and C: pericellular fibrosis are in minimal steatosis. A, B, D, and E: Azan Mallory staining. C and F: silver impregnation staining (Fibrosis is stained as if black yarns).

We further analyzed the progression to fibrogenesis in alcoholic rats. When groups of 3 rats received the CE2 diet, no fibrosis was induced in the course of several cycles of alternating weekly diets with VC (1 g/50 kgbw/d) or VB1 (25 mg/50 kgbw/d) or both VC and VB1 supplementation. Similar observations were made with groups of rats that received dietary supplements of VC, VB1 and $CaCl_2$ (0.63 g/50 kgbw/d). These results using the CE2 diet for basic feeding indicated that CE2 was too high in nutrients to achieve our goal.

In the next set of experiments, we used the vitamin-depleted atCE2 diet as the basic diet. When VC alone or both VC and VB1 were added, no fibrosis induction occurred. However, VC, VB1, and CaCl2 supplementation induced fibrosis (stained blue) in areas of steatosis (Fig. 4-A); faintly pericellular fibrosis was also noted (Fig. 4-B, C). In rats receiving VC, VB1, and CaCl2 supplementation, there was slightly less fibrosis than in rats receiving the alternating diets reported earlier (1).

We created a target protein expression system that induced fibrous histologic changes. We chose type I, III, and IV collagens as the target translational induction proteins (12-15). The specificity of the amino acid content of these 3 collagens, deduced from the PIR databases, was Proline (Pro) : Glycine (Gly) : Cysteine (Cys) = 1 : 1 : 0.2 (molar ratios). We decided to use L-proline (0.52 g/50 kgbw/d), glycine (0.4 g/50 kgbw/d), and L-cysteine (0.2 g/50 kgbw/d) to increase collagen translation on polysomes in the cytoplasm, and succeeded in inducing clear liver fibrosis in alcoholic rats (Fig. 4-D, E, F). The pathologic appearance was almost the same as that seen when the rats received alternating CE2 and atCE2 diets (1).

In our alcoholic rats drinking 70% ethanol (v/v), we found that the profilin (Fig. 3A and C) and type III collagen (Fig. 3A) mRNA levels were increased after intravenous injection of VC (1 g/50 kg). The animals were injected on the last day of atCE2 feeding (one week after completion of a VC-supplemented diet regimen). The effect of injected VC on ECM gene expression (fibronectin, Type IV collagen, b-actin) was negligible (Fig. 3B) within 24 hrs.

We hypothesize that following a chain reaction, the affected organ is in a state of acute distress and that an increase in profilin gene expression, efflux of profilin from injured cells, extracellular profilin-induced signal transduction of fibroblasts, expression of growth factor (b-FGF) (22), and excreted growth factor(s) stimulate fibroblasts.

Understanding of alcoholic fibrogenesis

The induction of alcoholic liver fibrosis in rats receiving standard diets was almost impossible before the development of our system. We trained rats to drink water containing from 12% to 70% ethanol. We modeled our high-concentration ethanol system on the historically validated experience of Vincent van Gogh who imbibed absinthe (70% ethanol). Van Gogh and Gauguin were sharing a small room in Arles, France. The evening before slicing off the lower half of his left ear incident they were at a Cafe drinking absinthe, a known epileptogenic drink that is now illegal. Our alcoholic patients, surveyed in psychiatric hospitals, also indicated that they preferred beverages with high alcohol concentrations (absinthe, vodka, shochu). We found it easy to train rats to imbibe the alcoholic beverages preferred by human alcoholics.

The program application to our animals was begun when the rats regularly imbibed water whose alcohol concentration exceeded 50% (Fig. 3A). We added 0.2% NaCl, 0.02% KCl to avoid salt deficiency due to dehydration. On alternating weeks, the rats received the vitamin-depleted diet. The autoclaved ($120^{o}C$ for 30 min) diet with depleted VC promoted the suppression of collagen gene set expression; depleted VA acted to suppress cell migration, and depleted VB1 suppressed cell activity via a small energy supply of ATP by decreasing oxidative decarboxylation in the TCA cycle and transketolase reactions at the pentose phosphate pathway. Our alcoholic rats never vomited the ingested food and liquid throughout our experiments. Without the added KCl and NaCl supply, our alcoholic rats did not survive.

Utilization of biological sensors to recognize metastasized cancer cells

To enable the fibroblasts to recognize migrating tumor cells, we used receptor ligand recognition systems that are known as biological sensors (Figure 5). Tumor cells express many cytokines and chemokines, e.g. IL-1, IL-6, IL-8, MIP1, TNFα, and TGFβ (23-27). As fibroblasts have many kinds of receptors for these cytokines and chemokines, we posited that it would be easy for them to recognize metastasized cancer cells (Fig. 5) (28-31).

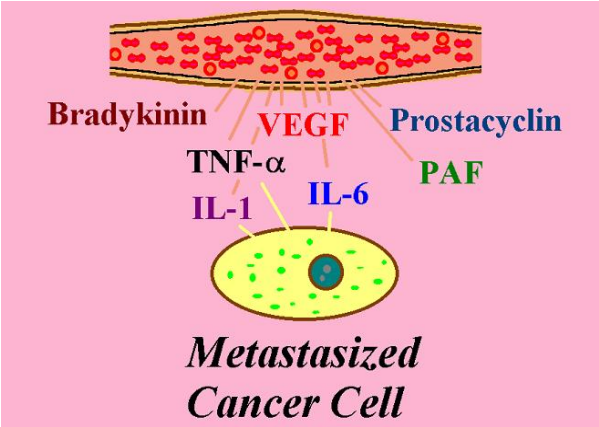


Figure 5. Our theory for enveloping metastasized cancer cells. The theoretical basis for applying our fuzzy set chaos algorithm to regulate histological reconstruction is shown.

Receiving commands from blood vessels around metastasized cancer cells

Bradykinin (32), vascular permeability factor (VEGF/VPF) (33) and other permeability proteins that are enriched around tumor cells, increase the efflux of acute-phase molecules including small molecular weight substances such as VC, VB1, VB2, VB6, VB12, and Ca2+ (Fig. 5). Thus, in gene expression programming projects, it is easy for surrounding fibroblasts to receive the flow of commands from the blood vessels.

Programming for gene and protein expression

For the purpose of gene expression regulation to induce fibrosis around metastasized tumor cells, we determined what kind of genes are involved. We divided the genes for fibrogenesis into 3 sets: the profilin gene set (Prof-gs) for emergency-phase mediation, the fibronectin gene set (FN-gs) for cell migration, and the collagen gene set (Col-gs) for fixation. We posited that artificially induced histological reconstruction was regulated by one-week cycles of fuzzy set ON/OFF switching of the fibrogenic gene set and that the weekly cycle had to be repeated over an extended period.

In this study we programmed the expression of gene sets by ON switching. The molecules we chose were target-gene set-specific. VC was introduced for Col-gs and Prof-gs expression. VB1 was used for ATP production as an energy supply. Calcium was used for activation of the calcium-binding domains of the elastin protein set (Ca2+B-SP) and/or calcium signal transduction system. Elastin connects collagen fiber bundles. Glycine, proline

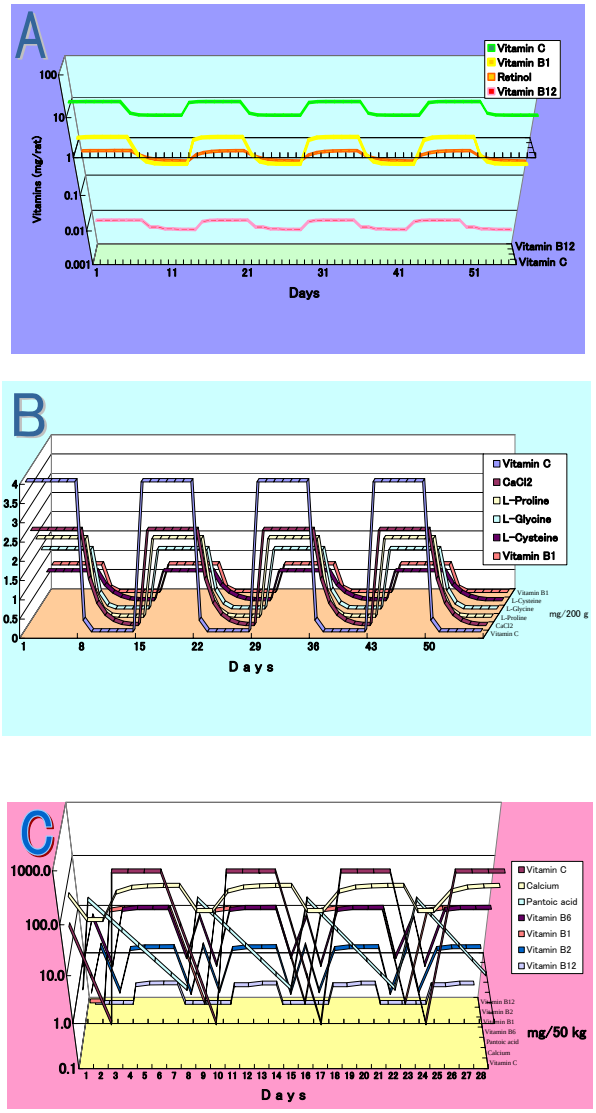


Figure 6. Process of programming for fibrosis. (A): Gene expression programming to induce fibrosis in rats fed alternating atCE2 and CE2 diets. (B): Advanced programming using alternating diets consisting of atCE2 and atCE2 supplemented with VC, CaCl2, VB1, Pro, Cys, and Gly. (C): Programming for envelopment of metastasized cancer cells in humans. The program used in cancer patients was designed precisely and cycled weekly. Prior informed consent was obtained from all cooperating patients.

We programmed to switch on collagen production and to inhibit competitively the other non-collagen proteins production, that leads metastasized cancer cells to signal transduction irregularities, which in turn confuses the translation of metastasized cells, resulting in their envelopment by fibroblasts.

and cysteine-enriched diets served to translate target collagen mRNAs preferentially. The diets were designed after comparative amino acid contents analysis of collagens and non-collagens. The protein enriched collagens served to express the collagen protein set (Col-SP) ON command, decreased collagen protein levels were used for the Col-SP OFF command.

Fig. 6-A indicates the initial program used for alcoholic rat fibrogenesis that simulated human alcoholics (1). The next program was set to induce fibrosis using selected molecules such as VC to switch ON the Col-gs and the Prof-gs in Fig. 6-B. Fig. 6-C depicts the enveloping program for human metastasized cancer cells.

The rainbow strategy

The technique we used to induced alcoholic fibrosis in rats was applied to envelope metastasized cancer cells with fibroblasts. If we could devise a way of enveloping metastasized cancer cells with fibroblasts, then we could possibly induce their death by denying them the nutrients required for their proliferation (Fig. 5). The commands for fibroblasts to envelope the target cancer cells were relayed via blood in blood vessels. To be applicable in humans, more precise and speedy expression programming would have to be deployed. Therefore, we used our alcoholic rat model to develop an advanced program of ON/OFF switching of the cycle in the course of a week. In humans, the medication changed daily, therefore, we called this the rainbow strategy because the packages containing the tablets were of 7 colors (red, orange, yellow, green, blue, indigo, and violet); one color for each day of the week (Table I).

Actual programming and command (molecules) roles extracted from databases for enveloping metastasized cancer cells by the GEP therapy

The program to envelop metastasized cancer cells by inducing fibroblasts to migrate around metastasized cancer cells involved the administration of VC (800 to 1000 mg/patient) and calcium lactate (1 g/patient) for 4 days, followed by 2 days without VC tablets and Ca (Table I). The withholding of VC served to initiate VD, VA, and VE relative increases that incompletely activate ECM-metalloproteinase, and to incompletely inhibit collagen synthesis. Thus, only the dietary VD, VA, and VE were able to convey plasticity to the envelope wall. We posit that the plasticity of the envelope wall prevents the sudden escape of metastasized cells through an occasional opening in the collagen-fortified wall envelope of a tumor mass. Simultaneously, VA begins to activate RARE-carrying genes called the FN-gs. Fibronectin directs post-mitotic fibroblasts to the weak part of the wall. This is essential because fibronectin binds DNA, actin, and integrins that are introduced into the exudate from broken cells.

Collagen gene activation was programmed on Wednesdays and at the same time, the calcium-binding domain of the elastin protein set (Ca2+B-SP) was activated by the calcium ion that plays a role in collagen fiber bundling. ECM fixes the fibroblasts tightly. Collagen, elastin, and calcium-enriched foods such as partially dried, steamed small fishes (SF:Pro: Gly:Cys:Ala = 14:17:4.8:21 mg/g vs sirloin steak: Pro:Gly:Cys:Ala = 7.4:8.1:2.2:11 mg/g) were made available in the diet to provide similar advantageous conditions for fibroblasts as does the collagen gene switched on by VC.

Mon	B_{12}	B_2	B_1	B_6	PA	Ca 130	VC	SF 15000
Tue	B_{12}	B_2 3.0	B_1	B_6 30	PA 100	Ca	VC	SF
Wed	B_{12}	B_2 0.5	B_1 25	B_6	PA	Ca 130	VC 800	SF 15000
Thu	B_{12} 0.25	B_2 3.0	B_1 25	B_6 40	PA	Ca 130	VC 800	SF 15000
Fri	B_{12} 0.25	B_2 3.0	B_1 25	B_6 40	PA	Ca 130	VC 800	SF 15000
Sat	B_{12} 0.25	B_2 3.0	B_1 25	B_6 40	PA	Ca 130	VC 800	SF 15000
Sun	B_{12} 0.25	B_2 3.0	B_1 25	B_6 40	PA	Ca 130	VC 800	SF 15000

Table I. Gene expression programming using our rainbow strategy. (33) VC, calcium (Ca), and small fishes (SF) enriched with skin [type I], bone [type I, III], blood vessels [type III], and lens capsule of the eye [type IV] were used as the source for collagen fiber production (ON switch collagen genes and collagen production). The major collagens contained are shown in brackets. SF were eaten on the day calcium was provided (5g SF is equivalent to 20 mg calcium). Ca was used for the activation of elastin, a calcium-binding protein. VB1, VB2, VB6, and nicotinamide were for ATP energy production and VB12 was for the proliferation of fibroblasts. The death of enveloped metastasized cancer cells is programmed to be forced by the withholding of nutrients. Pantothenic acid (PA) was used to prevent intestinal paralysis induced by the chronic administration of VB1. Doses are in mg/50 kgbw/d.

Furthermore, by administering VB1, VB2, VB6, and VB12, our program commands surrounding fibroblasts to enter mitosis under induced fibrogenesis.

By Monday of the second week, the envelope inflexibility is reduced and post-mitotic fibroblasts are induced to migrate to un-enveloped regions or delicate zones. This induces fibroblasts to envelop metastasized cancer cells smoothly and tightly but with some flexibility. The side effect of VB1 administration, paralysis of intestinal contractions (constipation), was prevented by administering pantothenic acid.

Judging the effect of gene expression programming on stopping the growth of metastasized cancer cells

Our 1st surviving patient, an 89-year old man (Table II) suffered from pathologically diagnosed scirrhous stomach cancer with signet ring cells. Death within one year from his malignant cancer was predicted by his attending surgeon who, based on his clinical experience, expected the development of metastasized tumors.

Case	Type of Cancer	GEP Therapy	QOL	Anti. C. A.	Radiation	Years	Lymph nod	s	Re-	Fate
♀51ys	Lung C (AC & SCC)	6 mth, 2 mth*	4(Pumpkin)	−/+/−/Iy	−−/+	1	Ope	3	+	†
♂87ys	Stomach C (scirrhus)	3 years	5	−	−	>5	Ope	0	None	alive
♀57ys	Intestinal Cancer	6 mth	5	+/−	−	>5	Endo Ope	\	None	alive
♀87ys	Lung C (small cell c.)	6 mth	5	−	−	>5	IAP,NSE	\	None	alive
♀64ys	Intest. & Stomach C	6 mth	5	+/−	−	>5	Ope	2	None	alive
♀44ys	Stomach Cancer	6 mth	5	−	−	>5	Endo Ope	\	None	alive
♀53ys	Stomach Cancer	6 mth	5	−	−	>5	Endo Ope	3	None	alive
♀49ys	Mamma Cancer	6 mth	5	+:txf/−	−	>5	Ope;Lymph V	8	None	alive
♂56ys	Intestinal Carcinoma	6 mth	4(Diarrea)	+:DFUR/−/+	−	5	Ope	2	+	†
♂74ys	Intestinal Carcinoma	8 mth	5	+:DFUR/−	−	>5	Endo Ope	0	None	alive
♀62ys	Choredochal Cancer	6 mth	5	−	−	>5	Ope	0	None	alive
♀67ys	Lung C (small cell c.)	8 mth	5	−	−	>5	ProGRP	0	None	alive
♂62ys	Pharingeal Carcinoma	8 mth	5	−	−/+	>5	Re-Ope	4	None	alive
♂56ys	Stomach Cancer	6+3 mth	5	−	−/+	4	Ope	0	None	alive
♂54ys	Stomach Cancer	6+3 mth	5	−	−/+	4	Ope	0	None	alive
♀60ys	Mamma Cancer	6+3 mth	5	+:txf/−	−	3	Ope;Lymph V	0	None	alive
♀62ys	Intestinal Cancer	6+3 mth	5	+:txf/−	−	2	High CEA	0	None	alive
♀72ys	Stmach Cancer	1 mth	5	+:txf	−	1	Ope:Lymph 0	0	None	alive

Table II. Effectiveness of the rainbow therapy for allowing gene expression programming. Operable cases with 3 (to 8) or less macroscopic lymph node metastases cured at present. ys: years old, type of cancer: stomach c, stomach cancer; lung c, lung cancer; small cell c: small cell carcinoma; intest: intestinal cancer; AC: adenocarcinoma; SCC: squamous cell carcinoma: bladder c: bladder carcinoma; GEP Therapy: period of gene expression programming therapy (mth: months, *: no GEP therapy from 7th-8th and from 10th-12th months); QOL: quality of life (5: excellent, 4: very good, 3: good, 2: bad, 1: worst), Anti. C. A.: Usage of Anti-cancer agent [-: no anti-cancer agent (antiCA), +/-: antiCAs were used during the first period, txf: anti-estrogen agent (tamoxifen), DFUR: doxifluridine, -/+/-/ly: no antiCA was used during the 1st quarter, antiCAs were used during the 2nd quarter and no antiCA was used during the 3rd quarter; immuno-therapy was administered by injecting lymphocytes from young healthy adults during the 4th quarter, +: antiCAs were used at the same time throughout]; Radiation: irradiation therapy (-: no irradiation, --+: irradiated in the last of 3 periods, -/+: irradiated in the second half-period); Years: years since GEP therapy, Lymph node, confirmation method of lymph node metastasis, s: number of metastasized lymph nodes by macroscopic inspection (Ope: original cancer and lymph nodes were removed and lymph node metastasis was counted, Endo Ope: operated using an endoscope, IAP, NSE: tumor marker IAP (immunosuppressive acidic protein) and NSE (neuron-specific enolase) were increased, Inope: inoperable, s: number indicates lymph nodes enlarged macroscopically by metastasis); Re-: Recurrence of cancer (None: no recur, +: recur, -: not cured), Fate: alive or died.

Another patient, a 90-year old woman was diagnosed with lung cancer (small-cell carcinoma); she had a dry cough and elevated tumor marker IAP and NSE. Her body temperature, tumor marker SCC (squamous cell carcinoma) and CRP (C-reactive protein: marker for infectious disease) levels were normal.

We were able to cure efficiently early and/or operable cases with metastasis to fewer than several lymph nodes as determined by macroscopic inspection. Metastasis to fewer than 3 lymph nodes (not pathological) seems to be the determinant of whether a patient can be cured or not at present. If we could induce the speedy envelopment of cancer cells by fibroblasts, we would be able to save patients with much more advanced cancers.

VA, VD, and VE-enriched foods have to be reduced to minimum levels because we noted that the daily intake of pumpkin or cheese reduced the programming effect. An advantageous situation for fibroblasts was created when VA, VD, and VE were decreased to below the usual level, because the function of VD, VE, and VA is activation of ECM metalloproteinase, inhibition of collagen synthesis, and induction of cell migration, respectively.

Chemotherapy and radiation treatment may affect the commands for gene expression programming. In our program, the possible effects of radiation and/or chemotherapy were not considered. In the future, it is necessary to evaluate gene expression programming in the presence of one or more of these therapies.

In non-operable cases, we were able to improve the patients' quality of lives (QOL). However, with time their tumor masses increased and organ damage led to their death. Our enveloping technique could stop the cough-related bleeding from lung metastasis.

The envelopment by fibroblast may not be able to save the lives of patients suffering from leukemia or benign tumors, and it may not be useful in non-operable cases or in patients with late-phase malignant tumors with metastasized foci receiving nutrients via the blood vessels. However, the treatment method described here seemed to improve the quality of the remaining life of these patients.

Based on the experience documented here, we suggest that metastasis of malignant tumor cells could be suppressed by using this expression programming after surgical removal of the original tumor mass and of suspicious lymph nodes.

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References and Notes

1. Hirano H, Hirano T, Hirata K, Tamura M, Yamaura T, Hamada T. Experimental liver fibrosis induced in rats receiving high doses of alcohol and alternating between regular and vitamin-depleted diets. Experientia. 1996 Jul 15;52(7):710-5.
2. Jimenez SA, Williams CJ, Myers JC, Bashey RI. Increased collagen biosynthesis and increased expression of type I and type III procollagen genes in tight skin (TSK) mouse fibroblasts.J Biol Chem. 1986 Jan 15;261(2):657-62.
3. Sullivan TA, Uschmann B, Hough R, Leboy PS. Ascorbate modulation of chondrocyte gene expression is independent of its role in collagen
4. secretion.J Biol Chem. 1994 Sep 9;269(36):22500-6.
5. Davidson JM, LuValle PA, Zoia O, Quaglino D Jr, Giro M. Ascorbate differentially regulates elastin and collagen biosynthesis in vascular smooth muscle cells and skin fibroblasts by pretranslational mechanisms.J Biol Chem. 1997 Jan 3;272(1):345-52
6. Bettendorff L, Wins P. Mechanism of thiamine transport in neuroblastoma cells. Inhibition of a high affinity carrier by sodium channel activators and dependence of thiamine uptake on membrane potential and intracellular ATP.J Biol Chem. 1994 May 20;269(20):14379-85.
7. Yamada KM, Aota S, Akiyama SK, LaFlamme SE. Mechanisms of fibronectin and integrin function during cell adhesion and migration.Cold Spring Harb Symp Quant Biol. 1992;57:203-12. Review. No abstract available.
8. Ruoslahti E. Fibronectin in cell adhesion and invasion.Cancer Metastasis Rev. 1984;3(1):43-51. Review.
9. Ruoslahti E. Fibronectin in cell adhesion and invasion.Cancer Metastasis Rev. 1984;3(1):43-51. Review.
10. Kim GS, Kim CH, Park JY, Lee KU, Park CS. Effects of vitamin B12 on cell proliferation and cellular alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells and UMR106 osteoblastic cells.Metabolism. 1996 Dec;45(12):1443-6.
11. Hirano H, Hirano T, Nakano M, Hirata K, Tamura M A novel approach for obtaining a cDNA clone that codes functional protein by applying the fuzzy set cloning theory.Biomedical Fuzzy and Human Science

2:19-24, 1996

12. Aso M, Tanaka Y, Miyata K, Hirano T, Yanagihara N, Etoh S, Hirano H cDNA Cloning of Inducer Reactive Protein in Adult T-Cell Leukemia Cell by Applying to The Fuzzy Set Cloning Theory for Sequencing Defuzzification.Biomedical Soft Computing and Human Sciences 5:15-20, 1999.
13. Nakamura H, Hirata K, Yamashiro K, Hiranuma K, Shibata K, Higashi K, Morita T, Hirano H. Increase of hepatic mRNAS of profilin, actin and extracellular matrix proteins after carbon tetrachloride treatment and partial hepatectomy in rats.Biochem Biophys Res Commun. 1994 Jan 28;198(2):568-73.
14. Hiranuma K, Suzuki K, Hirata K, Nakamura H, Higashi K, Hirano H. Extracellular matrices in peritendinous connective tissue after surgical injury to the chicken flexor tendon.Arch Orthop Trauma Surg. 1996;115(2):63-7.
15. Tamura M, Tanaka H, Hirano T, Ueta Y, Higashi K, Hirano H. Enhanced glomerular profilin gene and protein expression in experimental mesangial proliferative glomerulonephritis.Biochem Biophys Res Commun. 1996 May 24;222(3):683-7.
16. Hirata K, Nagata N, Hiranuma K, Hirano H, Osaka T, Itoh H, Ohsato K. The healing process of chronic colitis in rats, induced by 2,4,6-trinitrobenzene sulfonic acid, with special reference to the role of fibronectin.Scand J Gastroenterol. 1994 Jul;29(7):624-9.
17. Sanchez M, Arcella A, Pontarelli G, Gionti E. The role of cell adhesion in retinoic acid-induced modulation of chondrocyte phenotype.Biochem J. 1996 Jan 1;313 (Pt 1):201-6.
18. Vasios GW, Gold JD, Petkovich M, Chambon P, Gudas LJ. A retinoic acid-responsive element is present in the 5' flanking region of the laminin B1 gene.Proc Natl Acad Sci U S A. 1989 Dec;86(23):9099-103.
19. Kim RH, Shapiro HS, Li JJ, Wrana JL, Sodek J. Characterization of the human bone sialoprotein (BSP) gene and its promoter sequence.Matrix Biol. 1994 Jan;14(1):31-40.
20. Xiao G, Wang D, Benson MD, Karsenty G, Franceschi RT. Role of the alpha2-integrin in osteoblast-specific gene expression and activation of the Osf2 transcription factor.J Biol Chem. 1998 Dec 4;273(49):32988-94.
21. Lacraz S, Dayer JM, Nicod L, Welgus HG. 1,25-dihydroxyvitamin D3 dissociates production of interstitial collagenase and 92-kDa gelatinase in human mononuclear phagocytes.J Biol Chem. 1994 Mar 4;269(9):6485-90.
22. Tamura M, Tanaka H, Yashiro A, Osajima A, Okazaki M, Kudo H, Doi Y, Fujimoto S, Higashi K, Nakashima Y, Hirano H Expression of Profilin, an Actin-binding Protein, in Rat Experimental Glomerulonephritis and its Upregulation by Basic Fibroblast Growth Factor in Cultured Rat Mesangial Cells. J Am Soc Nephrol 11:423-433, 2000.
23. Punnonen R, Teisala K, Kuoppala T, Bennett B, Punnonen J. Cytokine production profiles in the peritoneal fluids of patients with malignant or benign gynecologic tumors.Cancer. 1998 Aug 15;83(4):788-96.
24. Lee LF, Li G, Templeton DJ, Ting JP. Paclitaxel (Taxol)-induced gene expression and cell death are both mediated by the activation of c-Jun NH2-terminal kinase (JNK/SAPK).J Biol Chem. 1998 Oct 23;273(43):28253-60.
25. Whitby D, Boshoff C. Kaposi's sarcoma herpesvirus as a new paradigm for virus-induced oncogenesis.Curr Opin Oncol. 1998 Sep;10(5):405-12. Review.
26. Woodworth CD, McMullin E, Iglesias M, Plowman GD. Interleukin 1 alpha and tumor necrosis factor alpha stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells.Proc Natl Acad Sci U S A. 1995 Mar 28;92(7):2840-4.
27. Abou-Shady M, Baer HU, Friess H, Berberat P, Zimmermann A, Graber H, Gold LI, Korc M, Buchler MW. Transforming growth factor betas and their signaling receptors in human hepatocellular carcinoma.Am J Surg. 1999 Mar;177(3):209-15.
28. Bird TA, Saklatvala J. Down-modulation of epidermal growth factor receptor affinity in fibroblasts treated with interleukin 1 or tumor necrosis factor is associated with phosphorylation at a site other than threonine 654.J Biol Chem. 1990 Jan 5;265(1):235-40.
29. Fini ME, Strissel KJ, Girard MT, Mays JW, Rinehart WB. Interleukin 1 alpha mediates collagenase synthesis stimulated by phorbol 12-myristate 13-acetate.J Biol Chem. 1994 Apr 15;269(15):11291-8.
30. Asao H, Takeshita T, Ishii N, Kumaki S, Nakamura M, Sugamura K. Reconstitution of functional interleukin 2 receptor complexes on fibroblastoid cells: involvement of the cytoplasmic domain of the gamma chain in two distinct signaling pathways.Proc Natl Acad Sci U S A. 1993 May 1;90(9):4127-31.
31. Lederer J, Czuprynski C. Characterization and identification of interleukin-1 receptors on bovine fibroblasts.Mol Immunol. 1993 Feb;30(2):191-6.
32. Wu J, Akaike T, Maeda H. Modulation of enhanced

vascular permeability in tumors by a bradykinin antagonist, a cyclooxygenase inhibitor, and a nitric oxide scavenger.Cancer Res. 1998 Jan 1;58(1):159-65.

33. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody.Proc Natl Acad Sci U S A. 1996 Dec 10;93(25):14765-70.

34. This rainbow strategy corresponds to machine language peculiar to human, and the following is the gene expression programming described by basic language.

```
' [ENVMETCAN.BAS]- Envelope Metastasized Cancer Cells-
*Start
  For Week=1 to 24
'- - - - Monday    - - - -
    Activate Ca2+ signal transduction system-set proteins (Ca2+ST-SP)
    If Found Profilin, Cytokines and Chemokines Then React Fibroblasts To These Molecules
'- - - - Tuesday - To prevent from intestinal paralysis caused by VB1 - - -
    Activate Peristalsis
'- - - - Wednesday - - - - Synthesize collagens - - - -
    Switch ON Profilin gene set (Prof-gs)
    Switch ON Collagen gene set (Col-gs) and Collagen protein set (Col-SP)
    Activate TCA-cycle
'- - - - from Thursday to Sunday - - - Synthesize collagens and fix them -
      For day=Thu    to Sun
        Fully Switch ON Prof-gs
        Fully Switch ON Col-gs and Col-SP
        Fully Activate TCA-cycle
        Proceed Mitosis
        Activate Elastin (Ca2+B-SP), Ca2+ST-SP and Proceed Fixation
      Next day
  Next Week
*End
```