Selfish Cells: Cancer as Microevolution

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Abstract

The properties of cancer result from microevolutionary selection pressures, which occur during the development and growth of damaged cells. Attributes of cancer cells include abnormal redox state, proliferation, anaerobic metabolism, immortality, aneuploidy, local invasion, and metastatic spread. Cancer cells differ sufficiently from those of their host, in terms of genetic makeup and competitive behaviour, to regard them as differing species, or "selfish" cells.

The microevolutionary model provides insights into the diversity of cancer cells; it also helps explain how these cells respond to treatment. In particular, it provides a scientific rationale for the role of antioxidant supplements in preventing cancer.

Introduction

Researchers have described carcinogenesis as the clonal proliferation of abnormal cells.[1] This paper extends the basic evolutionary model, placing cancer within a broad biological framework. We explain the development of cancer as the microevolution of damaged cells, resulting in the generation of selfish cells.

Cancer cells have lost their ability to cooperate with the body; instead, they evolve separately, like primitive, single-celled organisms.[2] Unlike healthy cells, cancer cells do not breed true, but exist as growing populations of diverse cells, subject to evolutionary selection.[3]

Redox mechanisms are crucial to the development of cancer. Carcinogens cause the release of oxidizing free radicals within the body; the resulting oxidation promotes cell division and facilitates the introduction of molecular errors. Such error-prone cell division is a necessary condition for carcinogenesis.[1]

Chromosomal abnormalities, including aneuploidy, are also central to the initiation of malignancy. Once growth has started, a tumour rapidly outstrips its blood supply.[4] As a result, tumour cells are generally short of oxygen and nutrients. Under these conditions, selection pressures favour cells with anaerobic forms of metabolism. Such redox factors allow us to explain why cancer is hard to eradicate and to clarify the mechanisms underlying various treatments.

Cancer Cell Characteristics

Malignant tumour cells, regardless of classification, have a consistent set of properties, which facilitate growth and provide selective advantages.

Specifically, cancer cells ignore signals to inhibit growth, and their apoptosis (cell suicide) control mechanisms are often faulty.[5] Most cancer cells are immortal, unlike many other body cells, which survive for a limited number of cell divisions.[6] In order for a tumour to grow to a significant size, some cancer cells are able to stimulate blood vessel growth, a process known as angiogenesis.[4] Malignant cancer is characterized by the ability to invade nearby tissues and form metastases; such cancer cells have an abnormal number of damaged chromosomes.[7] The cellular characteristics of cancer cells are similar to those of unicellular organisms.

Cell Cycle and Antioxidants

Oxidation and reduction are central to the microevolutionary model. The cellular redox state lies at the core of cell signalling mechanisms.[8] Cancer cells generally have an oxidizing internal state, with increased free radicals and lower lev-

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els of antioxidant enzymes.[7-10] Oxidation is intimately involved in the initiation of cancer,[11] as it facilitates cell division and maximizes growth. Cellular oxidation occurs early in cancer development,[12] and tissue oxidation is associated with development of cancer.[13] Tumour cells that develop an enhanced response to redox growth signals gain a competitive advantage and are able to flourish.[14]

Often, cancer cells achieve an elevated oxidation state by decreasing their levels of antioxidant enzymes.[15] Cancer-causing oncogenes can also act by increasing the level of oxidants.[16] A moderate increase in the level of oxidants, such as hydrogen peroxide and other reactive species, promotes cell growth and division.[17]

When healthy cells divide and become crowded, their growth is slowed by contact inhibition, which is associated with lower levels of oxidants within the cells.[18] Such contact inhibition may reflect the cells' internal redox state, corresponding to the sum of positive and negative growth signals.[18] As the oxidizing environment of a developing tumour prevents contact inhibition by redox signalling, cancer cells continue to multiply unhindered. However, since a reducing environment mimics the effects of contact inhibition signals, antioxidants may re-establish contact inhibition, thereby preventing cancer cell proliferation.

Compared to healthy cells, cancer cells are sensitive to exceptionally high levels of oxidation. This may be a consequence of their deficient antioxidant defences, particularly a lack of catalase.[19] A disturbed complement of antioxidant enzymes is characteristic of cancer.[12] Cancers are generally deficient in catalase and peroxidise, which break hydrogen peroxide down to oxygen and water.[20] Nearly all cancers are deficient in catalase, although the granular cell variant of human renal adenocarcinoma is a notable expection.[9]

This deficiency leaves the cells vulnerable to high levels of hydrogen peroxide. Beyond a certain point, therefore, increased oxidation becomes harmful to growing cancer cells. Intracellular production of high levels of oxygen species, such as hydrogen peroxide, slows proliferation of cells.[17] This further increase in oxidants can damage the cells, causing them to die by apoptosis.

A New Species of Selfish Cells

The development of cancer is a process that leads to the formation of new species of selfish cells, which compete with the host cells.[21] Malignant cells are aneuploid,[21] and possess the characteristics of growth, multiplication, mobility and invasiveness, consistent with a species invading a new environment.[22] In any setting, organisms sharing resources will tend to compete.

As a rule, similar organisms compete with each other more than do disparate ones. Individual cancer cells compete to leave more offspring than either neighbouring cells or host cells. According to Darwin's theory of natural selection, cells whose descendants become most numerous are biologically more fit.

Cell Proliferation with Error

The core requirement for microevolution of cancer is error-prone cell proliferation. Cancer development begins with increased cell division and genetic errors. The result is a population of increasingly abnormal cells, which are under selection pressure. They differ genetically from the healthy host cells and, when this difference is sufficiently great, their survival requirements are at odds with those of the host. At this point, we can describe the cells as cancerous. Cancer cells can eventually grow to out-compete the host cells.

A single faulty cell division, or a succession of such, as suggested by the traditional clonal evolution theory of cancer, is not enough to explain fully the massive

nuclear and genetic changes apparent in cancer cells.[21] Suggested mechanisms for such genome changes include damage to DNA repair mechanisms, and faulty cell division. Combined with microevolutionary selection pressure, such mechanisms could, over time, contribute to a varied population of proliferating cells. However, cancer develops within a period of only a few years. To produce a cancer cell by evolution, therefore, we require an almost instantaneous mechanism for speciation.

Aneuploidy

In biology, the generation of a new species may be considered a rare event, taking millions of years of evolution. Nevertheless, mechanisms exist for instantaneous speciation. The most common method of instant species formation is an increase in chromosome number, known as polyploidy. In plants, the rate of spontaneous duplication of chromosomes is similar to the mutation rate.[23] About half of all flowering plants could have originated by polyploid speciation.[24]

When healthy cells divide by mitosis, each daughter cell inherits the same number of chromosomes as the parent. By contrast, when cancer cells divide, the chromosome number can change. Malignant cells have non-standard numbers of chromosomes. The aneuploidy theory of cancer, which proposes that all cancer cells have irregular numbers of chromosomes,[24] originated over a century ago.[25]

An abnormal number of chromosomes may be a defining feature of human malignant cancer.[26,27] In a study of approximately 27,000 cancers, all had chromosomal aberrations.[28] Another survey found chromosomal variations in 2,400 cancers.[29]

Typical malignant cancers contain cells with multiple copies of damaged chromosomes.[30] The presence of an abnormal chromosome complement does not necessarily mean that a cell is cancerous, but cancer cells that are not aneuploid are exceptional.

Unlimited Cell Division

One way the body prevents cancer is by restricting number of times its cells can divide; this number is known as the Hayflick limit. Human fibroblasts cells, for example, can divide about 60 times. Aging is a side effect of this method of cancer prevention.[31] The Hayflick limit depends on the length of telomeres: highly repetitive regions of DNA at the end of a chromosome. With each cell division, the telomeres grow shorter until the chromosome becomes damaged and unstable. Unless the telomere is repaired, the cell will die.

The Hayflick limit restricts the life expectancy of damaged, yet dividing, cells. However, cancer cells are immortal. They contain telomerase, which repairs the telomeres, allowing the cells to divide endlessly.[32] Although all human cells have the potential to manufacture telomerase, they do not usually express these genes. Proliferating abnormal cells, however, are under selection pressure to activate the telomerase genes and extend their lifespan. Thus, cells that happen to contain active telomerase will have a selective advantage.

Early Tumour Growth

The growth of tumours involves increased cell division. In the early stages, lack of oxygen and nutrients restricts tumour growth. Proliferating cells form small tumours, which rapidly outstrip the available blood supply. The maximum achievable size is about that of a grain of rice, unless angiogenesis promotes the formation of new blood vessels. However, if a cancer cell evolves the ability to stimulate angiogenesis, the proliferating colony gains the potential for explosive growth. The mechanisms for angiogenesis exist,

unexpressed, in most cells. If damage to control mechanisms happens to activate these genes, the cell gains a substantial evolutionary advantage.

Larger Tumours

Once the cancer has gained the ability to stimulate blood vessel growth, it can expand rapidly. However, these new blood vessels can be abnormal or inefficient, thus they do not provide the same level of supply and drainage as normal vasculature. The necrotic centre of large cancers is lacking in oxygen and nutrients. Such tumours are stratified, with the outer region providing a relatively abundant environment. As the tumour grows, the habitat of its cells becomes more varied, providing selection pressure for increased diversity in the population. This increased cell and habitat diversity has implications for the effectiveness of treatment, as therapies that destroy cells from one part of the tumour may not work against those from a different part.

Anaerobic Metabolism

Throughout tumour development, cancer cells are under oxidative stress. Selection pressure favours cells that develop an anaerobic metabolism, depending on glycolysis and the use of glucose as an energy source. The idea that cancer cells are anaerobic has a long history, starting with Otto Warburg in the 1930s.

An early belief in the anaerobic metabolism of cancer cells led to the use of oxidant-based therapies; oxidation damage is now a primary element of many conventional cancer treatments. This metabolic approach led directly to the use of hydrogen peroxide as a treatment for the disease, in animal experiments and clinical studies.[33] Modern research is returning to this idea as an explanation for the cytotoxic actions of many substances, including ascorbate[34] and Motexafin Gadolinium.[35]

Glucose and ascorbate

Cancer cells have increased numbers of glucose transporters (GLUT) in their outer membranes.[36,37] The presence of these transporters allows modified glucose molecules, which enter cancer cells preferentially, to be used as radiological markers for cancer.[38] Besides glucose, these pumps also carry oxidized ascorbate, known as dehydroascorbate, into cells. Selection for cells with extra GLUT pumps is a consequence of the increased requirement for glucose, associated with survival pressures in an anaerobic habitat. Apart from the increased glucose uptake, this means that cancer cells can accumulate large amounts of dehydroascorbate. As cancer cells develop in an oxidizing redox environment, more local ascorbate will already be in the dehydroascorbate form.

The internal ascorbate concentration of cancer cells is proportional to the number of GLUT pumps. Since the rate constants for GLUT transport of glucose and dehydroascorbate are similar, cancer cells will accumulate large amounts of dehydroscorbate when either ascorbate is abundant in plasma or glucose levels are low.[39] We note that the increased absorption and utilization of ascorbate by cancer cells provides an explanation for the higher ascorbate bowel tolerance observed in these patients.

Ascorbate Concentration by Tumours

Cancer patients have low levels of vitamin C in their tissues, suggesting an increased requirement for ascorbate. Patients receiving conventional treatment have lower values still. The body's ascorbate reserves appear to be inversely related to the intensity of the treatment.[40] Hugh Riordan's research group describe an interesting example.[41] A 70-year old Kansas man, with cancer of the pancreas, received a 15-gram infusion of sodium ascorbate over a period of one hour. Immediately following the treatment,

his plasma vitamin C levels were lower than expected. Such low blood levels are consistent with the cancer absorbing and metabolizing more of the ascorbate than would normal tissues.

Cancer cells absorb higher levels of vitamin C than previously expected. Some contain specific ascorbate pumps, while others absorb dehydroascorbate.[42-44]

The mechanism in the latter type is comparable to that of specialised white blood cells, which oxidize vitamin C in their local surroundings, take up the resulting dehydroascorbate, and reduce it back to ascorbate.[45] Tumour cells accumulate high levels of ascorbate similarly.[46] Some cancer cells inhibit direct transport of vitamin C,[47] diminishing its antioxidant effects. However, when ascorbate molecules enter the environment of a tumour, they are oxidised to dehydroascorbate.[48] This can be taken up by glucose transporters in the outer cell membranes.[49,50] In animal models, tumour cells take up oxidised ascorbate rapidly.[51]

Malignant melanoma requires large amounts of glucose to grow rapidly. Some melanoma cells facilitate ascorbate transport by oxidation.[52] Such cells transport dehydroascorbate 10 times faster than do healthy melanocytes.[53] For comparison, both melanoma and healthy cells transport ascorbic acid with similar efficiency. The increased rate of dehydroascorbate transport is achieved using additional glucose transporters. Using these, the melanoma cells can concentrate dehydroascorbate to levels 100 times greater than the surrounding medium. High levels of glucose inhibit uptake of both dehydroascorbate and ascorbate.[54]

Cell Death

If animal cells become damaged or unregulated, they normally commit suicide. Such programmed cell death, or apoptosis, is triggered by a rise in oxidation levels. Ingeniously, the body uses redox signalling to initiate both cell division and cell suicide.[17,55] At lower levels, oxidation results in cell division. However, oxidation levels above a threshold cause apoptosis. By using the same signal to control both cell division and cell suicide, the cell gains a mechanism to destroy cancer cells that have lost their antioxidant controls.

During cell death by apoptosis, cells generate increased levels of oxidants, indicating their central role in the process.[56,57] A wide range of oxidants stimulate apoptosis, in a variety of cell types. Such oxidants include hydrogen peroxide, nitric oxide, redox-cycling quinones, oxidised low-density lipoproteins, lipid hydroperoxides, and diamide.[58,59] By varying the dose of such oxidants, researchers have induced a range of cell behaviours, ranging from increased cell growth, to apoptosis, through to catastrophic, uncontrolled cell death by necrosis.

It is possible to induce cell suicide using oxidants, even when oxygen levels are low.[60] Furthermore, cells that are short of oxygen may be more sensitive to free radicals.[61] Cells can eject glutathione, arguably the most important water-soluble cellular antioxidant, using molecular carriers in the surrounding cell membrane.[62] Such ejection induces redox stress in anaerobic cells.[63] The increased level of oxidation resulting from the loss of glutathione does not necessarily induce apoptosis, but can promote cell division, while simultaneously making the cell more sensitive to apoptotic signals.[64]

The ultimate cellular executioners are the mitochondria.[65] Enzymes in mitochondria appear to sense the level of oxidant species.[66] If the level of oxidants rises above a threshold, proteins leak out through the mitochondrial pores and the cell begins to die.[67] This process depends on the levels and nature of antioxidants in the cell.[68] If the energy in the cell is depleted, it may not be able to follow the

controlled pathway to suicide, and may be forced to die by necrosis.[69] Recently, scientists have described a third form of cell death, called autoschizis.[70] In autoschizis, cells die when the cytoplasm is expelled from the cell, leaving the nucleus apparently intact. Researchers discovered this form of cell death during an investigation of the redox-based, cytotoxic actions of vitamin K.

Many conventional cancer treatments, such as radiation and forms of chemotherapy, kill cells by inducing apoptosis. However, malignant cancer cells are often resistant to apoptosis, having evolved mechanisms to enable unconstrained growth. Ineffective treatments offer a selective advantage to apoptosis-resistant cancer cells. Follow up treatments will then also be ineffective, resulting in cancers that are multiply resistant to both drugs and radiation.

Treatment as Extinction and Population Control

Based on the microevolutionary approach, the purpose of cancer treatment is to eradicate cancer cells: a process analogous to the extinction of a species.

Existing knowledge on species extinction can be applied to this aim.[71] For example, diverse organisms are more difficult to eradicate than homogeneous ones. Large habitats, providing varied niches, select for robust organisms.

Widespread organisms are harder to drive to extinction than those in a single location. For instance, a species of bird on a single island may become extinct because of a volcano or human activities, such as tree felling or pesticide use.

The extinction of a species such as the rat, with a widespread intercontinental distribution involving varied habitats and behaviours, may be almost impossible to achieve, without destroying the terrestrial ecosystem of the planet.

Larger cancers are generally more diverse. Thus, cancers discovered late in development may be harder to destroy, because of increased cellular diversity.

Metastatic cancers are also more diverse, as they often comprise separate and distinct populations. This is analogous to the variation of species between islands and geographically distinct habitats. This increased diversity provides greater survival potential to a species and more resistance to extinction. For this reason, selective anticancer agents that are non-toxic to the host organism are most appropriate for cancer therapy. Moreover, we predict that such orthomolecular substances should be abundant in natural foods.[22]

Conclusions

This description of cancer, as the microevolution of precancerous cells resulting in new "species" of selfish cells, provides a fresh perspective on carcinogenesis. The model extends current ideas on the origins of cancer, including the multiple mutation, aneuploidy, and stem cell hypotheses. The microevolutionary model links the suggestion that cancer cells are anaerobic to more recent ideas, such as those involving redox signalling. Current models are consistent with the microevolutionary model, and can be seen as facets of it.

According to this model, cells that divide rapidly and do not breed true will eventually become cancerous. Evolutionary theory applies to any population of living cells that are subject to selection pressure. Consequently, there is no single cause for cancer: its features arise because of growing cells' consistent requirements for evolutionary success.

Increased variation in cancer cells, along with the related phenomenon of treatment resistance, is a direct consequence of microevolutionary selection. To flourish in the presence of selection pressures, cancer cells need to achieve

the proliferative and invasive properties of malignant cancer. However, the exact method used to achieve the malignant state will vary from one cancer to another. For example, one or more of a number of oncogenes, genes that stimulate cell division, may be activated to accelerate the growth of a cell. Alternatively, a similar growth increase can be achieved by inactivation of one or more tumour suppressor genes, which normally inhibit cell division. Either mechanism offers a selective advantage to the resultant, fast-dividing cancer cell.

Redox signalling and the levels of particular oxidants and antioxidants in cell compartments are central control factors in the cell cycle.[72,73] The redox state of cancer cells determines their growth, behaviour and death.[74,75] In the early stages, antioxidants can prevent cell division and allow redifferentiation of abnormal cells.[76,77] Cancer cells are often in a highly oxidizing redox state.[78]

As oxidant levels increase, the cell is driven towards cell suicide or apoptosis. The redox state of advanced and malignant cancers results in a balance between rapid cell division and apoptosis.

This model has direct implications for the prevention of cancer. A consistent feature of the initiation and development of cancer is cellular oxidation. Most known causes of cancer, including X-rays, inflammation and mutagenic chemicals, generate free radicals in the cell. While people cannot avoid all contact with such carcinogens, antioxidant supplements may provide a general mechanism for restraining the development of cancer. In a reducing environment, transformed cells can be made to redifferentiate. Establishment of such an environment would therefore inhibit cancer initiation and development. Antioxidants have been linked with cancer prevention for decades; the microevolutionary model clarifies these findings.

References

1. Fujii H, Marsh C, Cairns P, Sidransky D, Gabrielson E: Genetic divergence in the clonal evolution of breast cancer, *Cancer Res,* 1996; 56/7: 1493-1497.
2. Heppner GH, Miller FR: The cellular basis of tumor progression, *Int Rev Cytol,* 1998; 177: 1-56.
3. Pettit SJ, Seymour K, O'Flaherty E, Kirby JA: Immune selection in neoplasia: towards a microevolutionary model of cancer development, B*r J Cancer,* 2000; 8212: 1900-1906.
4. Folkman J: What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst,* 1990; 82/1: 4-7.
5. Carmody RJ, Cotter TG: Signalling apoptosis: a radical approach, *Redox Report,* 2001; 62: 77-90.
6. Kim NW, Piatyszek MA, Prowse KR, et al: Specific association of human telomerase activity with immortal cells and cancer, *Science,* 1994; 266/5193: 2011-2015.
7. Li R, Sonik A, Stindl R, Rasnick D, Duesberg P: Aneuploidy vs, gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy, *Proc Natl Acad Sci USA,* 2000; 97/7: 3236– 3241.
8. Smith J, Ladi E, Mayer-Proschel M, Noble M: Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell, *Proc Natl Acad Sci USA,* 2000; 9718: 10032-1007.
9. Oberley TD, Oberley LW: Antioxidant enzyme levels in cancer, *Histol Histopathol,* 1997; 122: 525-535.
10. Nindl G: Hydrogen peroxide-from oxidative stressor to redox regulator, featured review, *Cellscience Reviews,* 12, 2004.
11. Conour JE, Graham WV, Gaskins HR: A combined in vitro/bioinformatic investigation of redox regulatory mechanisms governing cell cycle progression, *Physiol Genomics,* 2004; 182: 196-205.
12. Bostwick DG, Alexander EE, Singh R et al: Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer, *Cancer, 2000;* 891: 123-134.
13. Hiroyasu M, Ozeki M, Kohda H, Echizenya M, Tanaka T, Hiai H, Toyokuni S: Specific allelic loss of p16 INK4A tumor suppressor gene after weeks of iron-mediated oxidativedamage during rat renal carcinogenesis, *Am J Pathol,* 2002; 160: 419-424.
14. Shackelford RE, Kaufmann WK, Paules RS: Oxidative stress and cell cycle checkpoint

function, *Free Radic Biol Med,* 2000; 28/9:1387-1404,
15. Oberley LW: Anticancer therapy by overexpression of superoxide dismutase, *Antioxid Redox Signal,* 2001; 3/3: 461-472.
16. Irani K, Xia Y, Zweier JL, et al: Mitogenic Signaling Mediated by Oxidants in Ras-Transformed Fibroblasts, *Science,* 1997; 275/5306: 1649-1652.
17. Burdon RH: Superoxide and hydrogen peroxide in relation to mammalian cell proliferation, *Free Radic Biol Med,* 1995; 18/4: 775-794.
18. Pani G, Colavitti R, Bedogni B, et al: A redox signaling mechanism for density-dependent inhibition of cell growth, *J Biol Chem,* 2000; 275/49: 38891-38899
19. Oberley TD: Oxidative Damage and Cancer, *Am J Pathol,* 2002; 160: 403-408.
20. Gonzalez MJ, Miranda-Massari JR, Mora EM, Guzman A, Riordan NH, Riordan HD, et al: Orthomolecular oncology review: ascorbic Acid and cancer 25 years later, *Integr Cancer Ther,* 2005; 41: 32-44.
21. Duesberg P, Rasnick D: Aneuploidy, the somatic mutation that makes cancer a species of its own, *Cell Motil Cytoskeleton, 2000;* 472: 81-107.
22. Hickey S Roberts H: *Cancer: Nutrition and Survival,* Lulu Press. 2005.
23. Ramsey J, Schemske DW: Pathways, mechanisms, and rates of polyploid formation in flowering plants, *Ann Rev Ecol Systematics, 1998;* 29: 467-501.
24. Sen S: Aneuploidy and cancer, *Curr Opin Oncol,* 2000; 121: 82-88.
25. Li R, Sonik A, Stindl R, Rasnick D, Duesberg P: Aneuploidy vs, gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy, *Proc Natl Acad Sci USA*, 2000; 97/7: 3236–3241.
26. Andreassen PR, Martineau SN, Margolis RL: Chemical induction of mitotic checkpoint override in mammalian cells results in aneuploidy following a transient tetraploid state, *Mutat Res,* 1996; 3722: 181-194.
27 Tucker JD, Preston RJ: Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment, *Mutat Res,* 1996; 3651-3, 147-159.
28. Mitelman F, Mertens F, Johansson B: A breakpoint map of recurrent chromosomal rearrangements in human neoplasia, *Nat Genet,* 1997 ; 4/15: 417-74. [Comments in *Nat Genet,* 1997 4/15 Spec No: 413 and *Nat Genet,* 1997 4/15; Spec No: 415-6]
29. Gebhart E, Liehr T: Patterns of genomic imbalances in human solid tumors, *Int J Oncol,* 2000; 162: 383-399.
30. Atkin NB, Baker MC: Are human cancers ever diploid—or often trisomic? Conflicting evidence from direct preparations and cultures, *Cytogenet Cell Genet,* 1990; 531, 58-60.
31. Hayflick, L: The limited in vitro lifetime of human diploid cell strains, *Exp, Cell Res,* 1965; 37: 614–636.
32. Kim NW, Piatyszek MA, Prowse KR, et al: Specific association of human telomerase activity with immortal cells and cancer. *Science,* 1994; 266/5193: 2011-2015,
33.Holman RA: The nature and functions of catalase, Mother Earth, *J Soil Assoc,* 1961; 6: 607-610.
34. Gonzalez MJ, Miranda-Massari JR, Mora EM et al: Orthomolecular oncology review: ascorbic acid and cancer 25 years later, *Integr Cancer Ther,* 2005; 41: 32-44.
35. Renschler MF: The emerging role of reactive oxygen species in cancer therapy, *Eur J Cancer,* 2004; 4013: 1934-1940.
36. Younes M, Lechago LV, Somoano JR, Mosharaf M, Lechago J: Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers, *Cancer Res,* 1996; 56/5: 1164-1167.
37. Smith TA: Facilitative glucose transporter expression in human cancer tissue, *Br J Biomed Sci,* 1999; 56/4: 285-592,
38. Flamen P, Stroobants S, Van Cutsem E, et al: Additional Value of Whole-Body Positron Emission Tomography With Fluorine-18-2Fluoro-2-deoxy-D-glucose in Recurrent Colorectal Cancer, *J Clinical Oncol,* 1999; 17/3: 894-901.
39. Krone CA, Ely JT: Controlling hyperglycemia as an adjunct to cancer therapy, *Integr Cancer Ther,* 2005; 41: 25-31.
40 Cameron E, Pauling L: *Cancer and Vitamin C,* Camino Books, Philadelphia. 1993.
41. Riordan HD, Riordan NH, Jackson JA, Casciari JJ, Hunninghake R, Gonzalez MJ, et al: Intravenous vitamin C as a chemotherapy agent: a report on clinical cases, *R Health Sci J,* 2004; 232: 115-118.
42. Vera JC, Rivas CI, Fischbarg J, Golde DW: Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. *Nature,* 1993; 364/6432: 79-82.
43. Rumsey SC, Kwon O, Xu GW, et al: Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid, *J Biol Chem,* 1997; 272/30: 18982-18989.
44. Rumsey SC, Daruwala R, Al-Hasani H, et al: Dehydroascorbic acid transport by GLUT4 in

Xenopus oocytes and isolated rat adipocytes, *J Biol Chem,* 2000; 275/36: 28246-28253.
45. Washko P, Yang Y, Levine M: Ascorbic acid recycling in human neutrophils, *J Biol Chem,* 1993; 26821: 15531-15535.
46. Langemann H, Torhorst J, Kabiersch A, et al: Quantitative determination of water-and lipid-soluble antioxidants in neoplastic and non-neoplastic human breast tissue, *Int J Cancer,* 1989; 43/6: 1169-1173.
47. Lutsenko EA, Carcamo JM, Golde DW: A human sodium-dependent vitamin C transporter 2 isoform acts as a dominant-negative inhibitor of ascorbic acid transport, *Mol Cell Biol,* 2004; 24/8: 3150-3156.
48. Baader SL, Bruchelt G, Trautner MC, Boschert H, Niethammer D: Uptake and cytotoxicity of ascorbic acid and dehydroascorbic acid in neuroblastoma (SK-N-SH) and neuroectodermal (SK-N-LO) cells, *Anticancer Res, 1994;* 141A: 221-227.
49. Nualart FJ, Rivas CI, Montecinos VP: Recycling of vitamin C by a bystander effect, *J Biol Chem,* 2003; 27812: 10128-10133.
50. Vera JC, Rivas CI, Zhang RH, et al: Human HL-60 myeloid leukemia cells transport dehydroascorbic acid via the glucose transporters and accumulate reduced ascorbic acid, *Blood,* 1994; 84/5: 1628-1634.
51. Agus DB, Vera JC, Golde DW: Stromal cell oxidation: a mechanism by which tumors obtain vitamin C, *Cancer Res,* 1999; 5918: 4555-4558.
52. Corti A, Raggi C, Franzini M, et al: Plasma membrane gammaglutamyltransferase activity facilitates the uptake of vitamin C in melanoma cells, *Free Radic Biol Med,* 2004; 3711: 1906-1915.
53. Spielholz C, Golde DW, Houghton AN, Nualart F, Vera JC: Increased facilitated transport of dehydroascorbic acid without changes in sodium-dependent ascorbate transport in human melanoma cells, *Cancer Res,* 1997; 5712: 2529-2537.
54. Malo C, Wilson JX: Glucose modulates vitamin C transport in adult human small intestinal brush border membrane vesicles, *J Nutr,* 2000; 1301: 63-69.
55. Laurent A, Nicco C, Chereau C, et al: Controlling tumor growth by modulating endogenous production of reactive oxygen species, *Cancer Res,* 2005; 65/3: 948-956.
56. McGowan AJ, Bowie AG, O'Neill LA, Cotter TG: The production of a reactive oxygen intermediate during the induction of apoptosis by cytotoxic insult, *Exp Cell Res,* 1998; 238: 248–256.
57. Slater AF, Nobel CS, Orrenius S: The role of intracellular oxidants in apoptosis, *Biochim Biophys Acta,* 1995; 1271: 59–62.
58. Lennon SV, Martin SJ, Cotter TG: Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli, *Cell Prolif,* 1991; 24: 203–214.
59. Sato N, Iwata S, Nakamura K, et al: Thiolmediated redox regulation of apoptosis, Possible roles of cellular thiols other than glutathione in T cell apoptosis, *J Immunol,* 1995; 154: 3194–3203.
60. Esposti MD, Hatzinisiriou I, McLennan H, Ralph S: Bcl-2 and mitochondrial oxygen radicals, New approaches with reactive oxygen species-sensitive probes, *J Biol Chem,* 1999; 274: 29831–29837.
61. Slater AF, Stefan C, Nobel I, van den Dobbelsteen DJ, Orrenius S: Signalling mechanisms and oxidative stress in apoptosis, *Toxicol Lett,* 1995; 82/83: 149–153.
62. van den Dobbelsteen DJ, Nobel CSI, et al: Rapid and specific efflux of reduced glutathione during apoptosis induced by anti-Fas/APO-1 antibody, *J Biol Chem,* 1996; 271: 15420–15427.
63. Ghibelli L, Fanelli C, Rotilio G, et al: Rescue of cells from apoptosis by inhibition of active GSH extrusion, *FASEB J,* 1998; 12: 479–486.
64. Fernandes RS, Cotter TG: Apoptosis or necrosis: intracellular levels of glutathione influence mode of cell death, *Biochem Pharmacol,* 1994; 48: 675–681.
65. Frade JM, Michaelidis TM: Origin of eukaryotic programmed cell death: a consequence of aerobic metabolism? *Bioessays,* 1997; 19/9: 827-832.
66. Blackstone NW, Green DR: The evolution of a mechanism of cell suicide, *Bioessays,* 1999; 21: 84–88.
67. Green DR, Amarante-Mendes GP: The point of no return: mitochondria, caspases, and the commitment to cell death. *Results Probl Cell Differ,* 1998; 24: 45–61.
68. Coppola S, Ghibelli L: GSH extrusion and the mitochondrial pathway of apoptotic signalling, *Biochem Soc Trans,* 2000; 28: 56–61.
69. Li P, Nijhawan D, Budihardjo I, et al: Cytochrome c and dATPdependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, *Cell,* 1997; 91: 479–489.
70. Jamison JM, Gilloteaux J, Taper HS et al: Autoschizis: a novel cell death, *Biochem Pharmacol,* 2002; 15/6310: 1773-1783.
71. Gatenby RA: Population ecology issues in

tumor growth, *Cancer Res,* 1991; 5110: 2542-2547.
72. Droge W: Free radicals in the physiological control of cell function, *Physiol Rev,* 2002; 82/1: 47-95.
73. Smith J, Ladi E, Mayer-Proschel M, Noble M: Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell, *Proc Natl Acad Sci USA,* 2000; 9718: 10032-1007.
74. Conour JE, Graham WV, Gaskins HR: A combined in vitro/bioinformatic investigation of redox regulatory mechanisms governing cell cycle progression, *Physiol Genomics,* 2004; 182: 196-205.
75. McEligot AJ, Yang S, Meyskens Jr FL: Redox regulation by intrinsic species and extrinsic nutrients in normal and cancer cells, *Ann Rev Nutr,* 2005; 25: 261-295.
76. Lupulescu A: The role of vitamins A, beta-carotene, E and C in cancer cell biology, *Int J Vit Nutr Res,* 1994; 63: 3-14.
77. Kenny PA, Bissell MJ: Tumor reversion: correction of malignant behavior by microenvironmental cues, *Int J Cancer,* 2003; 107/5: 688-695.
78. Mates JM, Sanchez-Jimenez FM: Role of reactive oxygen species in apoptosis: implications for cancer therapy, *Int J Biochem Cell Biol,* 2000; 32(2): 157-170.