

Biological Stoichiometry: An Ecological Perspective on Tumor Dynamics

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Biological stoichiometry is the study of the balance of energy and multiple chemical elements in biological systems. A key idea in biological stoichiometry is the growth rate hypothesis, which states that variation in the carbon:nitrogen:phosphorus stoichiometry of living things is associated with growth rate because of the elevated demands for phosphorus-rich ribosomal RNA, a requirement for rapid growth. In this article, we synthesize studies in the cancer literature to test the growth rate hypothesis; consistent with its predictions, rapidly growing tumors have elevated ribosome content, key oncogenes are closely affiliated with regulation of ribosome biogenesis, and tumor development has physiological impacts on patient phosphate metabolism. We also describe a new eco-evolutionary model of tumor dynamics that incorporates stoichiometric mechanisms. Since biological stoichiometry is fundamental in its approach, other areas of biology in which multiple key resources are involved in generating important tradeoffs may also benefit from the new tools provided by stoichiometric theory.

Keywords: cancer, growth rate, phosphorus, RNA, stoichiometry

Like many fields of the life sciences, cancer biology is an enormously complex and exponentially expanding field, involving work ranging from the molecular biology of oncogenes to environmental epidemiology. Cancer mortality rates are declining, primarily as a result of public health efforts (e.g., smoking reduction programs) that have reduced cancer incidence itself; however, survival rates for various types of cancers, once incurred, have shown only relatively modest improvements during recent decades (Clegg et al. 2002). For some cancers, such as lung cancer (Henschke et al. 1999, Patz et al. 2000), no changes in survival rates have been noted, despite recent advances in early detection. Thus, there is strong motivation to integrate diverse fields of knowledge in cancer biology and to introduce new conceptual and theoretical frameworks that might improve researchers' understanding of tumor dynamics, so that better therapeutic measures might be developed. Of particular importance is the production of mechanistically based predictive models of tumor dynamics that can abstract meaning from the forest of molecular biological details about oncogenesis and tumor progression. Developing such models would help researchers focus on the functionally critical aspects of tumors most deserving of attention. Although it is certainly true that cancer is a multifaceted disease with a variety of proximate triggers in different tissues and in different patients, there is also a strong possibility that cancers share a central functionality

arising from the common cellular machinery on which all cells rely for their proliferation (Hanahan and Weinberg 2000). If so, then more generalized frameworks for modeling cancer dynamics might be possible. Furthermore, understanding this core commonality might better allow researchers to identify fundamental tradeoffs experienced by tumors, which in turn could permit more effective therapy. These issues call attention to the need for a greater integration of diverse realms of life sciences, from genomics to ecology; indeed, this may be the major challenge for biology in the 21st century (Vogel 1998, Wilson 1998, Hanahan and Weinberg 2000, Michener et al. 2001, Thompson et al. 2001, Cottingham 2002).

Recent work in the fields of ecosystem ecology and life-history evolution has produced a set of ideas and an analytical framework, known as "biological stoichiometry," that we believe exhibit such cross-disciplinary integration. To illustrate the potential utility of successfully synthesizing diverse biological knowledge, we attempt to show that this stoichiometric framework has strong relevance for understanding tumor biology. Our main objectives are (a) to introduce the central concepts of biological stoichiometry to

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a general audience of biologists who may not have encountered them in the ecological literature and (b) to review empirical findings from cancer studies as an example of how these ideas might be applied in arenas outside of ecology. We propose a viewpoint in which tumor and host are seen as a coupled ecological system, each with particular material demands that establish the terms of the interaction and thus affect its dynamics. The goal in cancer therapy is to ensure that the host (the patient) wins in this ecological competition (that is, the tumor is eliminated) or, at least, that there is a long-term stable coexistence in which the host maintains an acceptable level of health (that is, damage to normal tissue is minimized). By applying a stoichiometric perspective to represent the multivariate material demands and transactions of the players, health care professionals might be better able to turn the tables of competition in the patient's favor. To gain such a perspective, researchers need to understand the functional ecology of the evolving tumor in its host habitat.

Biological stoichiometry: What is it and where did it come from?

Biological stoichiometry is the study of the balance of multiple chemical elements in biological systems (Elser et al. 2000a). It is an extension of the theory of ecological stoichiometry, an approach developed in ecosystem ecology to better understand ecological dynamics in terms of the material balance of interacting organisms in the environment (Reiners 1986, Elser and Urabe 1999, Sterner and Elser 2002). The development of ecological stoichiometry has been motivated by the realization that different organisms can contrast strongly in their elemental composition, with particular attention to the macroelements carbon (C), nitrogen (N), and phosphorus (P). In lakes, for example, some crustacean zooplankton species have low body P content (< 0.5 percent P by dry weight; atomic ratios of C:P = 200, N:P = 40), while others have high P content (> 1.5 percent P; C:P = 67, N:P = 13). It is now known that these differences have major implications for the ecology of these organisms (Sterner and Elser 2002). In particular, P-rich animals are unusually sensitive to the P content of their food, suffering strong declines in growth and reproduction when consuming food with low P content, and are vulnerable to erratic population dynamics and possible extinction in environments that do not supply sufficient P to allow them to achieve a stable population equilibrium. Thus, the relative stoichiometric requirements of a species appear to be a key aspect of its ecological niche (figure 1a, bottom).

These major ecological implications of body C:N:P stoichiometry have motivated ecologists to ask why, at both the proximate physiological and the evolutionary levels, different taxa or growth stages are characterized by contrasting C:N:P ratios (Elser et al. 1996, 2000a). While in some biota (autotrophic organisms like plants) biomass C:N:P ratios closely mimic the relative C, N, and P supplies of the environment, in other biota (e.g., metazoans and heterotrophic bacteria) C:N:P ratios are tightly regulated by homeostatic

feedbacks. Thus, it seems likely that biomass C:N:P ratios are relatively stable characters that relate to the overall life history of the organism involved. A primary hypothesis under consideration by evolutionary ecologists interested in stoichiometric patterns is the growth rate hypothesis (Elser et al. 2000a). In this hypothesis (figure 1a), high P content in biomass (low C:P and N:P ratios) is caused by increased biomass allocation of P-rich ribosomal RNA (rRNA), which is necessary to achieve rapid rates of growth or development. This implies that taxa that have evolved high growth rates with high P demands are more likely to face ecological constraints caused by insufficient supplies of P from the environment or diet and, thus, that there is an unavoidable tradeoff in the evolution of a rapid growth-rate strategy. Empirical evidence supporting the growth rate hypothesis is accumulating (Elser et al. 1996, 2000a, 2000b, Main et al. 1997, Vrede et al. 1998).

Researchers are also seeking to understand the genetic underpinnings of the growth rate hypothesis. Elser and colleagues (2000a) propose that particular differences among biota in the structure of ribosomal RNA genes (rDNA), in terms of copy number and aspects of the intergenic spacer region, are associated with the ability to produce the high rRNA phenotype necessary for rapid growth. In particular, Elser and colleagues note, it is commonly seen in selection experiments and cross-species comparisons that rapidly growing taxa have unusually long intergenic spacers in their rDNA repeats. This trend is associated with an increased number of promoter sequences, which results in increased rDNA transcription. This has recently been confirmed for *Daphnia* in an artificial selection experiment (Gorokhova et al. 2002) that also documented a direct connection to differences in RNA levels and, uniquely, in P content. As reviewed by Elser and colleagues (2000b), existing studies also indicate that rapidly growing, high-RNA biota have greater numbers of copies of the rDNA cistrons, either in the normal genome or via extra chromosomal amplification. In sum, this work highlights rRNA and associated genes as central components in the evolution of rapid growth. These associations have important ecological implications, both for stoichiometric food quality (P-rich animals need P-rich food) and for nutrient recycling feedbacks in the ecosystem (P-rich animals tend not to liberally recycle P to the environment).

We now turn to applying the concept of biological stoichiometry, and more particularly the growth rate hypothesis, to tumor biology. Since tumors are generally tissues with abnormally high growth rates, and since malignancy is generally proportional to tumor proliferation rate, cancer biology provides an attractive arena to test various aspects of the growth rate hypothesis. For the same reasons, stoichiometric theory as developed in ecology may be able to offer important insights into factors regulating the outcome of the interaction between tumor and host. We address two sets of questions. First, are increased rRNA levels and rates of rRNA production and ribosome biogenesis associated with tumorigenesis? What genetic mechanisms in the rDNA and in the regulatory pathways leading to the rDNA are associated with

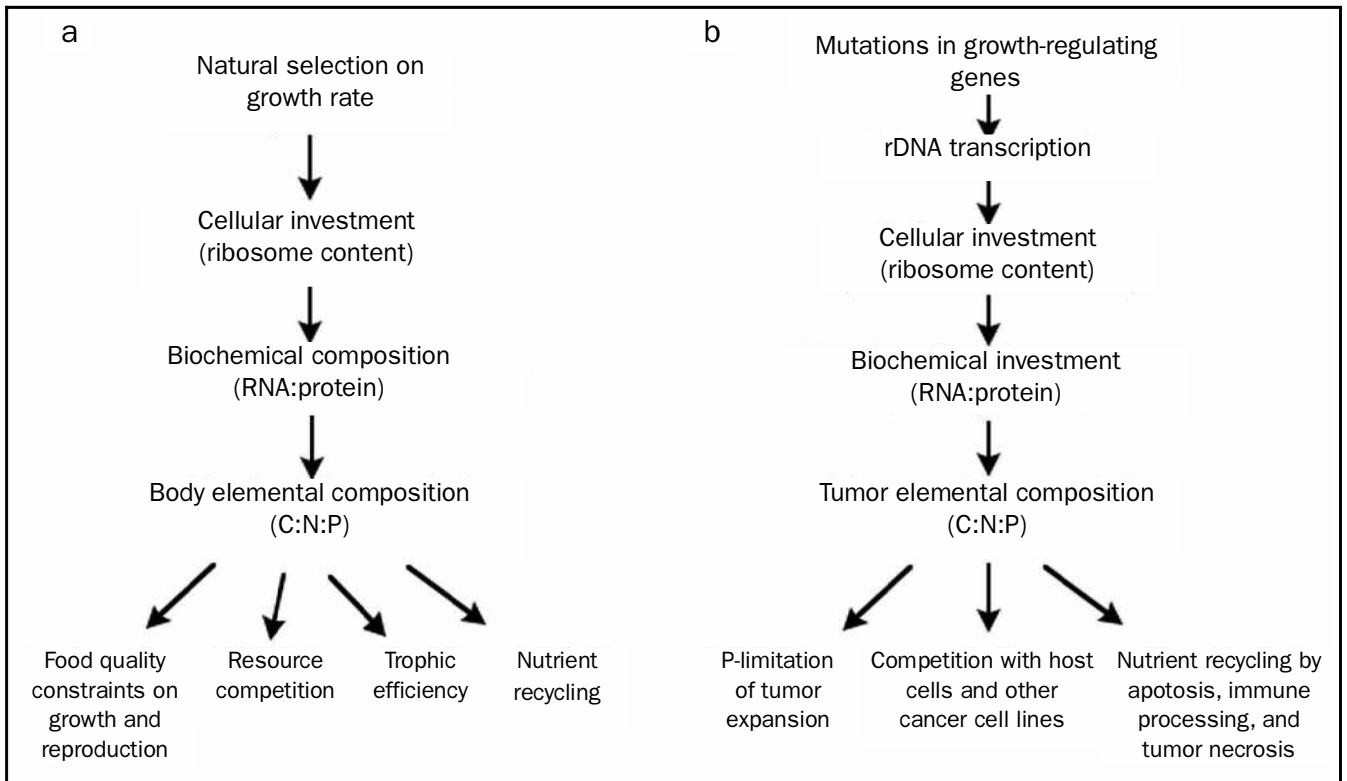


Figure 1. The stoichiometric growth rate hypothesis as applied to (a) evolutionary ecology and (b) cancer biology. In ecology, the ramifications of organismal carbon:nitrogen:phosphorus (C:N:P) stoichiometry for ecological interactions (indicated at the bottom of figure 1a) are now reasonably well established (Stern and Elser 2002), while the biochemical and evolutionary determinants of organism C:N:P ratios are only beginning to be illuminated (Elser et al. 2000a). With respect to cancer biology, the ideas and prior findings synthesized in this article suggest that the stoichiometric approach may be plausibly applied to better understanding of tumor dynamics as well.

tumor development and proliferation? Are important oncogenes connected to the machinery of cellular proliferation? Second, is there any evidence indicating that tumors have unusually high P demands (as a result of high RNA levels) or that there is an association between P metabolism and cancer development in humans or model systems? The evidence we will summarize in these sections generally supports the growth rate hypothesis as applied to tumors. Therefore, we will conclude with a description of how more functionally realistic models of tumor dynamics might be constructed using stoichiometric principles. Finally, we will draw out some tentative implications of these ideas for the prevention and treatment of cancer.

Biological stoichiometry in tumors: Cellular proliferation, ribosome biogenesis, and genetic pathways leading to cancer

A key component of the growth rate hypothesis is that rapidly growing cells, tissues, and organisms have elevated allocations to ribosomal RNA (in this article, "allocation" and "content" are used interchangeably to refer to the fraction of the total dry mass of the cell or organism given over to that component; Elser et al. 1996, 2000b). For example, in rapidly growing cultures of *Escherichia coli*, at least 30 to 40 percent of

total cell dry mass can be allocated to RNA; as in most growing cells, the vast majority (> 85 percent) of this is rRNA (Maaloe and Kjeldgaard 1966, Sutcliffe 1970). Therefore, since the RNA:protein ratio of a prokaryotic ribosome is about 2:1 by weight, ribosomes can constitute more than 50 percent of total biomass of rapidly growing *E. coli* cells (using the 40 percent total RNA value and assuming 85 percent rRNA, about 35 percent of total dry mass would be contributed by rRNA and 17.5 percent by ribosomal proteins). High levels of RNA that dominate biomass P pools are not only observed in microorganisms. In many metazoa, trajectories of ontogenetic development involve periods of rapid growth, especially early in the life cycle. These rapid growing stages are generally characterized by increased levels of total RNA allocation, as has been observed for the fruitfly *Drosophila melanogaster* (Church and Robertson 1966) and the aquatic crustacean *Daphnia magna* (McKee and Knowles 1987). Cross-species comparisons also support this pattern. For example, the slow-growing crustacean *Bosmina longirostris* has significantly lower RNA levels (2 to 3 percent RNA) than the rapidly growing crustacean *Scapholeberis mucronata* (15 to 20 percent RNA; Dobberfuhl 1999). Importantly, these taxa also differ significantly in P content (< 0.9 percent P versus about 2.5 percent P).

Do such patterns hold for tumor cells, especially when comparing RNA and ribosome levels with those of the normal, differentiated tissues in which the tumor cells are developing? We suggest that they do, at least for cancers characterized by rapid cellular growth rates, and we propose an alternative version of the growth rate hypothesis for application in cancer biology (figure 1b). Various lines of evidence, some well established and some quite new, strongly support the idea that RNA content is elevated in cancer cells and that genetic events leading to cancer are often directly or indirectly linked to ribosome biogenesis. The cases reviewed by Darzynkiewicz (1988), and more recently by Ruggero and Pandolfi (2003) with an emphasis on genetic changes, include these examples:

- In murine skin tumors induced by epidermal application of tumor promoters, the RNA:DNA ratio and RNA content (percentage of dry mass contributed by RNA) were 2 to 3 times higher than in normal tissues (De Young et al. 1977).
- In several types of leukemia, cellular RNA content of lymphocytes was strongly correlated with accelerated cellular growth kinetics and ultimately with patient prognosis (Darzynkiewicz 1988).
- In a study of gynecological cancers in which neoplastic tissues were compared with their normal counterparts, DNA content and RNA content in the neoplastic tissues were increased 1.6- and 2.4-fold, respectively (Chu et al. 2002).
- Similarly, cellular RNA content was increased by a factor of 1.4 in *myc*-transfected neuroblastoma cells relative to normal cells (Boon et al. 2001).
- In a study of breast cancer, assay of tumor DNA content was of little utility, but RNA content correlated well with tumor grade, histological type, hormonal status, and patient survival (ElNaggar et al. 1996).

An interesting medical example of a converse problem lies in anemia (idiopathic ineffective erythropoiesis [IIE]), in which the concern is related to *insufficient* rates of cellular proliferation (in this case, of red blood cells). Patients suffering from IIE had erythroblasts containing only about 70 percent of normal levels of rRNA (Lourenco et al. 1978). Thus, in a situation of excess cellular proliferation (aggressive cancers), rRNA levels are amplified, while in a situation of insufficient cellular proliferation (anemia), rRNA levels are depressed.

Cancer biologists have also shown that expression of rDNA and ribosomal protein genes is associated with tumor development and eventual outcome. Ribosomal genes are actively expressed in the area of the nucleus known as the nucleolus. Various cytological studies show that in cancer cells the nucleolus is enlarged because of increased transcriptional activity and that its size and shape are predictive of cellular proliferation rate and patient prognosis (Derenzini et al. 1994, 1998). In an analysis of microarray data from medulloblastomas,

three of the four messenger RNAs (mRNAs) that were identified as most useful in predicting treatment outcome coded for ribosomal proteins (Pomeroy et al. 2002). These investigators also showed that cancerous cells with elevated expression of these ribosomal protein genes had higher ribosome contents.

The preceding overview reiterates what cancer biologists already know: Ribosome biogenesis and oncogenesis are closely connected. But what of the specific genetic mechanisms involved? The literature on cancer genetics is immense. Here we wish only to highlight a few studies illustrating a point that has been made by others (Hanahan and Weinberg 2000, Ruggero and Pandolfi 2003): Genetic alterations that are associated with cancer development very frequently involve changes in growth-signaling pathways that lead to the rDNA. Here are some examples:

- p53 (perhaps second only to rubisco [ribulose biphosphate carboxylase/oxygenase] as the world's most studied protein) is a well-known tumor suppressor whose activity is lost or mutated in more than half of all human tumors (Hickman et al. 2002). It is known to repress transcription of RNA polymerase III (pol III; Cairns and White 1998), which is responsible for production of small rRNAs and tRNA (transfer RNA). p53 may also repress RNA polymerase I (pol I; Zhai and Comai 2000, but see Budde and Grummt 1999), which transcribes genes for the larger rRNA units.
- The *myc* family of oncogenes has been shown to be active in regulation of nucleolin (a nucleolar protein directly involved in ribosome biogenesis) and BN51 (a cofactor of RNA pol III; Greasley et al. 2000). Further, serial analysis of gene expression in *myc*-transfected neuroblastoma cells showed that a large majority of the 114 up-regulated genes were associated with ribosome production (Boon et al. 2001). It has been known for some time that overexpression of *c-myc* protein leads to increased cellular proliferation (e.g., Gu et al. 1993).
- In ovarian tumors, a transcription factor (TFIIIC2) for RNA pol III is overexpressed (Winter et al. 2000); this is sufficient to stimulate pol III transcription in cell extracts. Thus, changes in TFIIIC2 expression resulting from impacts of tumor viruses or carcinogens may contribute to cellular transformation.
- The mode of action of cisplatin, a widely used chemotherapy agent, has been shown to involve inhibition of *in vivo* synthesis of ribosomal RNA by redistributing UBF (upstream binding factor) in the nucleolus and thus inhibiting RNA pol I transcription and preventing production of large rRNA subunits (Jordan and Carmo-Fonseca 1998).
- Genomic amplification of retrotransposons in the rDNA intergenic spacer has been shown to be associated with tumorigenesis, nucleolar activity, and tumor stage in Hodgkin's lymphoma cell lines (MacLeod et al. 2000).

- After p53, retinoblastoma genes (genes coding for the “pocket” proteins pRb, p107, and p130) are possibly the best-studied genes in cancer biology (Hickman et al. 2002). These proteins are potent growth inhibitors that are now known to operate by interfering with transcription by pol I and pol III (Ciarmatori et al. 2001) by binding and inactivating UBF. (Recall that pol I and pol III are responsible for production of ribosomal RNAs.)
- Various studies have linked overexpression of the laminin receptor precursor (LRP) with tumor progression. This protein has recently been found to be highly conserved across taxa and is multifunctional, playing a role as a component of the small ribosomal subunit (Ford et al. 1999). Thus, LRP’s association with tumor proliferation appears to have nothing to do with laminin processing per se; rather, it is just one of many genes entrained in support of the elevated ribogenesis demands of tumor cells. Perhaps other oncogenes will be found to have a similar “camouflaged” role in ribosome production.

This list barely scratches the surface of a massive literature emerging from cancer genetics. Although there are other important aspects in the genetic progression to cancer (e.g., evasion of programmed cell death or apoptosis; Hanahan and Weinberg 2000), the work discussed provides support for genetic aspects of the growth rate hypothesis in highlighting a close connection between cellular proliferation and rRNA production on rDNA. But do these links carry through to the level of cellular elemental composition, as predicted by the growth rate hypothesis?

Biological stoichiometry in tumors: A key role for phosphorus?

Up to this point we have argued that the growth rate hypothesis, as developed for evolutionary ecology, also applies to cancer. Indeed, as we have just shown, previous studies on cellular proliferation, RNA and ribosome content, and rDNA regulation in tumors seem quite congruent with the growth rate hypothesis. A final step will be to find out whether tumors have unusually high demands for P because of the need to generate and maintain increased levels of P-rich ribosomal RNA. While there are innumerable studies on tumor genetics and many studies on tumor RNA levels, the elemental composition of tumors has been little studied and their overall P content even less so. Thus, the evidence here is somewhat more tenuous; we merely spotlight some provocative findings indicating that such differences may indeed exist and that more studies are warranted.

Some evidence for increased levels of P in tumors comes from ³¹P nuclear magnetic resonance studies. For example, in a study of human breast cancer, total phosphate in tumor tissues decreased significantly during successful treatment (Leach et al. 1998). This study also determined that significant quantities of P were associated with phosphomonoesters, phosphodiesteres, and nucleoside triphosphates; these moieties would be expected to be closely involved in nucleic acid

metabolism. Some studies have considered broad swaths of the periodic table in chemical comparisons of tumor and normal tissues but have managed to omit P (e.g., Ng et al. 1997). However, some researchers have included P in their investigations and indeed report higher levels of P in tumors (Durak et al. 1994, Garg et al. 1994). Indeed, lymphoblast cells have P content that is five times higher than that of normal lymphocytes (Bourke and Yanagawa 1993), and thus treatment of acute leukemia can induce hyperphosphatemia when that P is mobilized into body fluids as phosphate (Milionis and Elisaf 1999). The converse has also been observed: A patient with acute malignant lymphoma experiencing blastic crisis presented hypophosphatemia, during which time white blood cells had greatly elevated P content (Perek et al. 1984). The patient then experienced hyperphosphatemia after receiving chemotherapy. Despite the apparent significance of the tumor P pool in cancer patients, at present the data on P content in tumor cells are heterogeneous and are generated by a variety of methods. Systematic studies using standardized methods are needed. Nevertheless, the data in general suggest that tumors often have elevated requirements for key elements and especially for P. Furthermore, this elevated demand for P appears to have clinical significance. Can researchers take advantage of this information to improve their understanding of tumor dynamics and to build more realistic models of tumor dynamics?

Biological stoichiometry in tumors: A modeling strategy

Stoichiometric modeling is beginning to transform the theory of food-web ecology (Sternner and Elser 2002). This is occurring because stoichiometric models of ecological interactions capture key mechanisms that are absent from less realistic models; in doing so, they generate novel outcomes more in keeping with actual dynamics of ecological systems. The key advance of these models is to move beyond a parameterization in which state variables are assumed to be composed of a single substance (e.g., biomass, C, energy) to one in which state variables are viewed as being composed of more than one thing. Including even one additional currency (for example, depicting organisms as mixtures of C and P) has major quantitative and qualitative impacts on model dynamics (Andersen 1997, Loladze et al. 2000). Modeling of tumor development is a growing field in cancer biology; however, no one, to our knowledge, has constructed a stoichiometric model of a growing tumor. Such an effort is now under way (Kuang et al. 2004).

Our approach builds on existing models of tumor dynamics by incorporating the effects of the vascular system in delivering materials to the host tissue and developing tumor, but this approach is distinguished by incorporating simple stoichiometric constraints on the growth of genetically distinct tumor cells. In this sense, it is a stoichiometrically explicit Darwinian model of tumor growth, treating the organ with a tumor as a heterogeneous community with physiologically distinct cell types. Adding an ecological

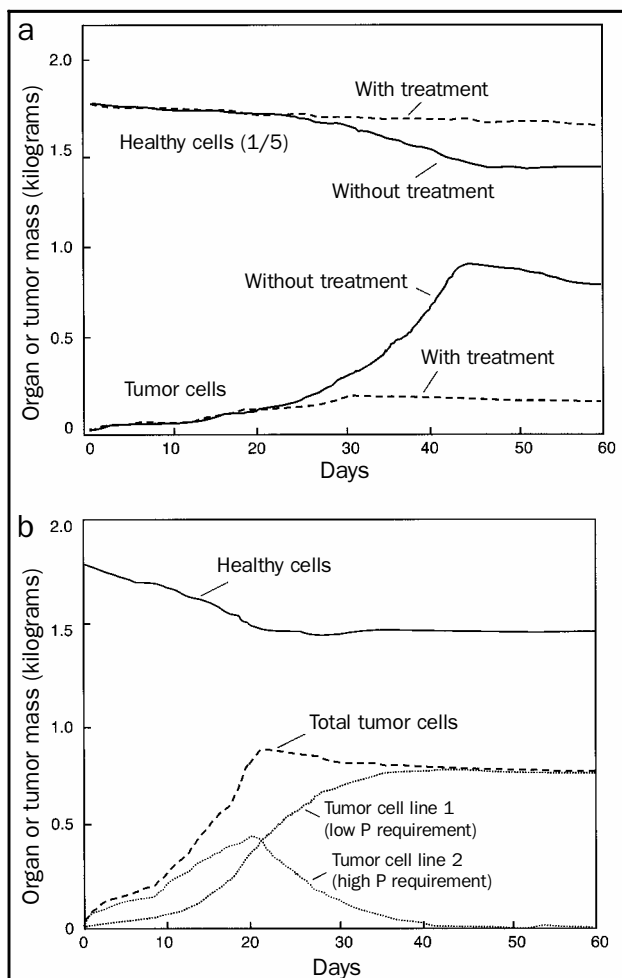


Figure 2. Tumor dynamics in a stoichiometric model. The model framework includes a formulation for a time-delayed response of angiogenesis; a set supply of phosphorus (P) that can be partitioned among organ tissues, vascular tissues, and the tumor; and the possibilities of modeling a single homogeneous tumor as well as heterogeneous tumors with cell lines having different physiological properties. The models start with an assumption that they are following a tumor in an average-sized human (with a body weight of about 70 kilograms [kg], or just over 154 pounds) and that the tumor has reached a detectable size (0.01 kg [0.35 ounces]) and is already vascularized. Phosphorus is brought to the organ at a constant rate, assuming normal dietary P intake and uniform distribution in the circulatory system. (a) Effects of reducing the ability of a homogeneous tumor to acquire P from the blood supply by a factor of two. Note that this twofold reduction in the tumor's supply of P leads to a fourfold reduction in steady-state tumor mass. (b) Dynamics of a heterogeneous tumor with two cell types, one (tumor cell line 2) having a higher growth rate and higher P requirement than the other (tumor cell line 1). In this simulation, the high-P cell line starts with a higher abundance than the low-P line. Note that, despite its lower growth rate, the tumor line with lower P content eventually outcompetes the other line. Source: Kuang and colleagues (2004).

dimension with stoichiometric tradeoffs has important ramifications for the tumor dynamics of the model (figure 2). In particular, for a realistic range of parameter values and initial conditions, the model identifies consistent constraints on tumor growth resulting from limitations set by P supply, suggests potential therapeutic approaches that limit tumor access to the limiting P (figure 2a), and predicts intense competition between tumor cell lines differing in their specific P requirements.

Of particular interest is the result that, as in ecological models of interspecific competition (Tilman 1982), tumor cell lines with low P requirements (in the parlance of ecological competition models, low "minimal cell quota") outcompete those with higher P requirements at steady state (figure 2b). That is, cell lines bearing mutations that promote fast growth (and that concomitantly impose higher P requirements) can dominate early in tumor development, before intense resource competition sets in. However, as the tumor becomes larger and its growth is constrained by delivery of limiting resources, fast-growing cell lines with high cell quotas for P suffer disproportionate growth reductions and begin to be outcompeted by lines with lower P requirements. This implies that faster-growing tumor lines, which may be more malignant, are at an inherent competitive disadvantage in a P-limited environment. This may help in understanding the tendency of different cancers to metastasize. This model remains oversimplified, and a more realistic treatment of various processes, such as programmed cell death and immune system-mediated mortality, may eventually be required. However, it may have some advantages over existing models of tumor dynamics, which do not account for the multiple material demands of tumor and host cells. It will be of particular interest to determine whether different types of tumors have differing stoichiometric signatures and to assess whether this knowledge is useful in better predicting their dynamics in model settings and in reality. A more general point from this exercise is that all biological processes involve transactions with multiple key constituents (energy and various organic and inorganic materials); it is likely that their dynamics will be better described by formulations that encompass the multivariate character of their resource requirements.

Biological stoichiometry in tumors: Medical implications

A variety of clinical implications emerge from applying a stoichiometric perspective to tumor dynamics. First, knowing that a growing tumor has a disproportionate requirement for a key element, such as P, provides a potential target for designing tumor-targeted drugs. An example from infectious disease is perhaps applicable here. It is recognized that pathogenic organisms often have unusually high demands for iron (Smith 1993). This knowledge has recently been used to design "Trojan horse" drugs that use the high iron affinity of the pathogen to slip cytotoxins selectively into the disease organism (Miller et al. 2001). Perhaps P-limited

tumor cells produce high-affinity PO_4 (phosphate) transporters to improve their ability to acquire scarce PO_4 from the intercellular milieu. Drugs capable of closely interfering with those transporters might be designed, or genetic tools might be used to prevent transcription of genes coding for high-affinity PO_4 transporters. Alternatively, a Trojan horse delivery system might be designed to capitalize on the potential P hunger of tumor cells.

Application of stoichiometric theory may help improve the effectiveness of chemotherapy. Chemotherapy commonly achieves a 1000-fold reduction in the tumor population before the tumor gains resistance to the treatment, but it is essential to drive the tumor population to extinction to achieve a cure. Making matters worse, the extracellular environment subsequent to chemotherapy may instead only encourage, rather than discourage, proliferation of surviving tumor lines, given the apparently large amounts of PO_4 (and other materials) liberated when tumor cells are killed by chemotherapy. One can imagine ways in which this might be managed. For example, chemotherapy might be followed by dialysis in which blood plasma is selectively stripped of circulating PO_4 . This would hinder the proliferation of surviving tumor lines and make it easier for them to be managed by the immune system, resulting in negative population growth rates and eventual extinction.

A stoichiometric perspective may also aid in improving dietary approaches to cancer management. For example, osteomalacia is a serious problem in some late-stage cancer patients. In osteomalacia, patients experience bone loss and symptoms of PO_4 deficiency, resulting from elevated rates of PO_4 loss in urine. It appears that osteomalacia is caused by a compound released by the tumor, which reduces reuptake of PO_4 in the kidney (Nelson et al. 1996); the resulting hypophosphatemia is generally treated by intravenous or oral PO_4 supplementation. Thus, osteomalacia can be seen as a manipulation (*sensu* Nesse and Williams 1994) of the host by the tumor to satisfy its massive P demands by mobilizing stores of PO_4 from the patient's bones. That PO_4 depletion is an outcome of the cancer and not the result of its treatment is supported by studies of bone mass at diagnosis in children with acute lymphoblastic leukemia (Halton et al. 1995). In that study, postdiagnosis patients entered therapy with low bone mass and abnormal mineral homeostasis. A stoichiometric view, therefore, suggests an alternative approach to nutritional support of the cancer patient. Massive PO_4 supplementation of the patient, while addressing immediate symptoms, may only preferentially "feed" the tumor. A strategy that could be more effective would be taking measures to raise the patient's ability to form bones and retain bone mass: for example, supplementing the patient's diet with appropriate levels of vitamin D. However, since renal PO_4 loss would continue, some supplementation of PO_4 would still be required. Stoichiometric theory suggests that such supplementation might best involve joint dietary manipulations that shift overall resource ratios (*sensu* Smith 1993) to favor the patient over the tumor. Stoichiometric analysis indicates

that fast-growing organisms suffer disproportionately when resources are supplied at high C (energy):P ratios (Sterner and Elser 2002). This suggests that an optimal diet for a cancer patient might be one that supports bone formation by supplying sufficient PO_4 (and calcium, along with vitamin D) but also accompanies the P with disproportionately high levels of readily available energy (C). Fructose, which is known to reduce plasma phosphate levels (Hallfrisch et al. 1986), may be an ideal C (energy) source for such an application. These ideas, of course, remain conjecture and in no way represent clinical or dietary recommendations; we provide them only to illustrate how stoichiometric thinking can potentially open new avenues for developing therapeutic approaches.

Conclusions

Recent pleas have been issued for conceptual and theoretical approaches to make functional sense of the intimidating mass of information surrounding cancer etiology and dynamics (Hanahan and Weinberg 2000, Gatenby and Maini 2003). The same can probably be said of many unresolved problems in biological medicine. The situation is unlikely to improve much in the face of the blizzard of data emerging from high-throughput sequencing machines and microarray readers. This challenge is similar to the daunting prospect faced by ecologists and evolutionary biologists in confronting the factors influencing the vast biodiversity of living species present in nature and in connecting that diversity to the functioning of those species in food webs and biogeochemical cycles. Just as ecologists cannot incorporate all biological diversity in their theoretical models, but must instead focus on key interactions that capture most of the major mechanisms, cancer theory cannot include all the genetic and protein diversity underlying tumor biology. Instead, cancer biologists must develop conceptual clarity and theoretical tools of intermediate complexity to identify key mechanisms (Gatenby and Maini 2003). Perhaps the same conceptual framework, biological stoichiometry, that now helps ecologists understand ecosystem dynamics will be of use to cancer biologists or to others working to confront the immense biological complexity emerging in the era of genomics.

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