Evolution of cooperation among tumor cells

Evolution of cooperation among tumor cells

Robert Axelrod*†, David E. Axelrod‡, and Kenneth J. Pienta§

*Gerald R. Ford School of Public Policy and Department of Political Science, University of Michigan, Ann Arbor, MI 48109; ‡Department of Genetics and Cancer Institute of New Jersey, Rutgers, The State University of New Jersey, Piscataway, NJ 08854; and §Departments of Internal Medicine and Urology, University of Michigan Medical School, Ann Arbor, MI 48109

Contributed by Robert Axelrod, July 19, 2006

The evolution of cooperation has a well established theoretical framework based on game theory. This approach has made valuable contributions to a wide variety of disciplines, including political science, economics, and evolutionary biology. Existing cancer theory suggests that individual clones of cancer cells evolve independently from one another, acquiring all of the genetic traits or hallmarks necessary to form a malignant tumor. It is also now recognized that tumors are heterotypic, with cancer cells interacting with normal stromal cells within the tissue microenvironment, including endothelial, stromal, and nerve cells. This tumor cell–stromal cell interaction in itself is a form of commensalism, because it has been demonstrated that these nonmalignant cells support and even enable tumor growth. Here, we add to this theory by regarding tumor cells as game players whose interactions help to determine their Darwinian fitness. We marshal evidence that tumor cells overcome certain host defenses by means of diffusible products. Our original contribution is to raise the possibility that two nearby cells can protect each other from a set of host defenses that neither could survive alone. Cooperation can evolve as by-product mutualism among genetically diverse tumor cells. Our hypothesis supplements, but does not supplant, the traditional view of carcinogenesis in which one clonal population of cells develops all of the necessary genetic traits independently to form a tumor. Cooperation through the sharing of diffusible products raises new questions about tumorigenesis and has implications for understanding observed phenomena, designing new experiments, and developing new therapeutic approaches.

carcinogenesis | hallmarks | tumorigenesis | cancer

The evolution of cooperation has a well established theoretical framework based on game theory (1–6). This approach has made valuable contributions to a wide variety of disciplines, including political science, economics, and evolutionary biology. Two kinds of cooperation have been recognized: commensalism, in which one individual of a pair benefits but not the other; and mutualism, in which both benefit, resulting in synergy. In each case, new properties may emerge in a cooperating group that the individuals do not exhibit.

Existing cancer theory suggests that tumors are monoclonal, i.e., they develop from a single cell that starts to divide to form a tumor mass because of an initiating carcinogenic event. The initiated cell does not have all of the necessary mutations (genetic and epigenetic) to form a population of fully malignant cancer cells. As these cells are exposed to further promotional events and divide, errors in DNA replication result in daughter cells, or subclones, that are genetically different from each other, resulting in tumor cell heterogeneity. Prevailing theory suggests that, as these distinct subclonal populations of cancer cells continue to divide, they evolve independently from one another, and one subclone acquiring all of the genetic traits or hallmarks necessary to form a population of fully malignant cancer cells. It is well recognized that this is an inefficient process, with many of the subclones dying because they are genetically unstable or do not contain a set of mutations that sustain viability in the face of host defenses.

Here, we add to this theory by regarding tumor cells as game players whose interactions help to determine their Darwinian fitness. We marshal evidence that genetically distinct tumor cells cooperate to overcome certain host defenses by exchanging different diffusible products. Our original contribution is to raise the possibility that two nearby subclones can protect each other from a set of host defenses that neither could survive alone, potentially speeding the process of tumorigenesis through the more rapid emergence of malignant populations of cells that contain all of the necessary hallmarks of cancer (Fig. 1). We therefore propose that tumor progression may be facilitated by the evolution of cooperation in the form of by-product mutualism among genetically diverse tumor cells. Our hypothesis supplements, but does not supplant, the traditional view of carcinogenesis, in which one subclone of cells evolves independently to acquire all of the necessary genetic traits to form a tumor. Cooperation through the sharing of diffusible products raises new questions about tumorigenesis and has implications for observed phenomena, designing new experiments, and developing new therapeutic approaches.

Examples of cooperation have been found among a wide range of organisms, from viruses to animals to humans (1–4). It is important to realize that cooperation is not limited to sentient organisms. Cooperation may occur among organisms such as viruses and cells that do not have intent, emotions, sophisticated memory, or any of the other attributes unique to humans or even mammals. A player's strategy is what it does as a function of what it can respond to (although, as will be shown, even this contingent action is not always needed). Two or more players interact, and the payoff for each is influenced by what they all do.

Evolutionary biology now uses game theory to understand the origin, spread, and maintenance of cooperation. The evolutionary interpretation of game theory uses standard Darwinian principles: individuals that interact with each other and their environment, phenotypes that are heritable, change in heritable genotypes by mutation and other mechanisms, competition among individuals for limited resources, and selection. The criterion for selection is fitness, i.e., an increase in the number of progeny by sexual or asexual (clonal) reproduction.

Most studies of the evolution of cooperation deal with the apparently paradoxical situation of altruism, i.e., cases in which the benefits to the recipient are costly to the donor. The classic setting for the study of cooperation with altruism is the "Prisoner's Dilemma." In scenarios such as the Prisoner's Dilemma, individual "defectors" have a competitive advantage over cooperators, so the evolution and maintenance of cooperation represent a puzzle to be explained. This puzzle has most often been resolved by pointing to the individual's ability to make its cooperation contingent in a manner that favors either close relatives or those who reciprocate help (6, 7).

Not all forms of cooperation require altruism. For example, in by-product mutualism, two or more individuals provide help to each other simply as a consequence of each maximizing its own

Conflict of interest statement: No conflicts declared.

Freely available online through the PNAS open access option.

Abbreviation: GF, growth factor.

†To whom correspondence should be addressed. E-mail: axe@umich.edu.

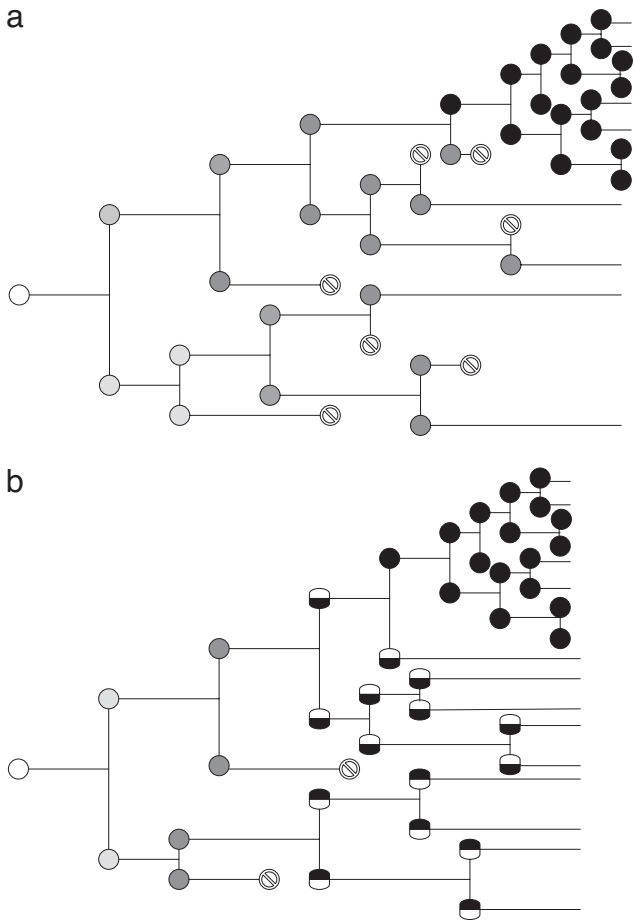

Fig. 1. Tumor progression can reach full malignancy before any one cell accumulates each of the necessary mutations. (*a*) Traditional view of tumor progression: Competition. Genetically unstable partially transformed cells (●) proliferate. The cells compete for limited oxygen, essential nutrients, and GFs; therefore, many die (⦸). Eventually, one cell accumulates sufficient mutations to express all of the functions required for a clone of fully malignant cells to emerge (●). (*b*) Hypothesis of tumor progression: Cooperation. Genetically unstable partially transformed cells (●) proliferate and yield different mutant cell types (◓, ◒). The different cell types cooperate with each other, enabling them to survive and proliferate. The concept of cooperation among partially transformed cells is added to the traditional view of tumor progression. As in the traditional view, eventually one cell may accumulate sufficient mutations to express all of the functions required for a clone of fully malignant cells to emerge (●). An example of cells that cooperate by producing different GFs is shown in Fig. 2.

fitness. We hypothesize that mutualism exists between different partially or fully transformed tumor cells in a heterogeneous mixture of cells.

Current Perspective on Cancer

Cancer is currently viewed as the result of the accumulation of mutations within a genetically unstable heterogeneous population of cells, with the eventual emergence of a malignant subclone that has accumulated all of the functions necessary, in solid tumors, for invasion, metastasis, and defeating the host's defenses (8). Each subclonal population of cells evolves independently from the others, competing for space and resources such as oxygen and nutrients. Although cancer is known to be composed of a spectrum of diseases that involves a large number of genotypes and phenotypes, it is the accumulation of mutations in a clone of cells that is a single and unifying theme for all cancers (ref. 9; Fig. 1). The minimum set of genotypes/

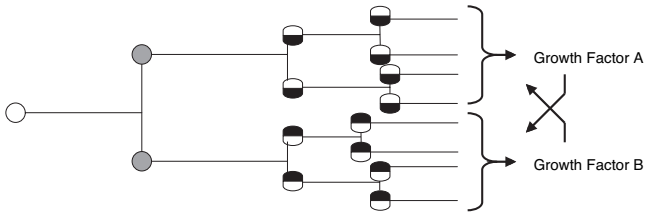

Fig. 2. Intratumor cooperation can occur among partially transformed mutant cells that have complementary needs, such as two different GFs. Some cells (◒) produce only GF A, and other cells (◓) produce only GF B, but together they produce both GFs. Crossfeeding is a form of cooperation that enables each cell type to survive and proliferate.

phenotypes that a cancer cell must acquire to become malignant has been called "the hallmarks of cancer" (10). These include: (*i*) self-sufficiency of growth signals, (*ii*) insensitivity to growth inhibitor signals, (*iii*) evasion of programmed cell death, (*iv*) limitless replicative potential, (*v*) sustained angiogenesis, and (*vi*) tissue invasion and metastasis (10). Cells that have accumulated some, but not all, of these hallmarks or other changes necessary for malignancy are referred to here as "partially transformed."

Although single random mutations are usually considered rare events, the acquisition of these survival traits is a result of the accumulation of random mutations in genetically unstable cells that eventually result in a malignant cancer with the ability to metastasize and kill the host (10, 11). Specific genetic mutations may contribute only partially to the acquisition of a single hallmark capability or may confer several hallmark capabilities at once.

Tumor-cell heterogeneity is the result of genetic or epigenetic instability, the inherent property of cancer cells that results in the creation of multiple daughter cells or subclones with different properties (phenotypes). Some of the progeny cells may be defective and die because of chromosome nondisjunction or apoptosis. Others may be more robust, resulting in the outgrowth of a subclone with new features, such as the ability to invade and metastasize.

Our hypothesis of cooperation among tumor cells, as outlined below, suggests that some tumor cells may provide limited resources that other cells require, allowing for the survival of more cells and the subsequent accumulation of mutations faster than traditional theory allows. The mass of the tumor is made up of heterogeneous populations of tumor cells, and one of these subclones eventually acquires the "full deck" of mutations that result in one or more cells with the ability to successfully metastasize to distant sites.

It is also now recognized that tumors are heterotypic, with cancer cells interacting with normal stromal cells within the tissue microenvironment, including endothelial, stromal, and nerve cells. This tumor cell–stromal cell interaction in itself is a form of commensalism, because it has been demonstrated that these nonmalignant cells support and even enable tumor growth (9, 12–16). The traditional view of cancer is evolving into one of a tumor being viewed as a complex interaction between cancer cells with the surrounding stroma, with the extracellular matrix, and with the host's defense systems within the tumor microenvironment. The hypothesis of cooperation among tumor cells outlined here adds to this traditional view the possibility (even likelihood) that partly transformed cells interact and cooperate with each other to form a malignant tumor. For example, it is possible that a partially transformed subclone will have acquired all of the hallmarks except two sharable ones, growth factor (GF) A and GF B (Fig. 2). If a cell acquires the ability to produce GF A, it still requires GF B, and it will not yet proliferate rapidly. However, once either that cell or a nearby cell acquires GF B, it

will have all of the hallmarks of a successful cancer cell, even though one is shared, and malignancy will be established. It is likely that one of the millions of cells within the diffusion range of GF A will acquire GF B before the relatively few cells with GF A can acquire GF B.

The Hypothesis of Cooperation Among Tumor Cells

Our hypothesis is that, as a population of cells undergoes transformation to a fully malignant state, there exists the opportunity for this heterogeneous population of partially and fully transformed tumor cells to cooperate with each other; that malignancy can be an emergent property of the cooperating population of these cells. This interaction of tumor cells is in addition to the interaction of tumor cells with nontumor host cells. This hypothesis does not replace the existing view of cancer as a monoclonal expansion of one initial cell but rather offers an explanation of how the growth of subclones can be supported by neighboring partially or fully transformed subclones through sharable resources. We suggest that the application of evolutionary cooperation theory can provide new insights into cancer.

Two levels of evolution need to be distinguished. The first is the evolution of the host species, in which the relatively slow time scale is measured in decades per generation. At this level, the host species evolves mechanisms, such as the immune system, which can destroy cells that have become selfish and proliferate inappropriately; for example, those that have mutated and have become independent of growth inhibitory signals. There is also the fast time scale on which individual cells proliferate, measured in hours or days. Our focus will be on the fast time scale. We assume (as in humans) that a variety of defenses against cellular selfishness have already evolved, and we don't deal with their future evolution, which takes thousands of years. We focus on the fast (small) level of evolution that takes place in less than the generation time of the host. We use the standard interpretation of evolution at the cellular level; the "individuals" are cells, the mutations are in the cells, and the selection is at this level, because some subclones outproliferate others (17–19).

We apply cooperation theory to the evolution of "selfish" cells. Partially transformed cells can evolve to be a population of fully transformed tumor cells interacting with each other as well as with stromal cells of the microenvironment to form the malignant tumor. The hypothesis of cooperation among partially transformed tumor cells includes the concept that there can be a proliferative advantage for these cells within the tumor mass, which the individual cells would not have if they did not benefit from cooperation. Although it has been previously demonstrated that cooperation occurs between tumor and stromal cells, we hypothesize there is also cooperation among partially transformed tumor cells.

Cooperation among partially transformed cells may take the form of by-product mutualism by sharing resources. For instance, one subclone could produce paracrine GFs that the other requires and visa versa (Fig. 2). Cooperation can also take the form of commensalism, in which only one subclone benefits. For instance, a subclone that induces vascular GFs could provide oxygen and nutrients to other subclones that are not angiogenic.

The traditional view is that individual subclones within a tumor must accumulate all of the hallmarks of cancer to develop despite the host's defense mechanisms. However, cooperation theory suggests that individual cells are not required to accumulate all of the hallmarks to proliferate within the tumor environment. Rather, cells that have accumulated only some of the hallmarks may cooperate with other partially transformed tumor cells to form a community that has the necessary phenotypes required to grow as a malignant tumor.

Cooperation may account, in part, for the frequency of cancer. Consistent with the traditional view of cancer, eventually a clone can develop a full deck of mutations (Fig. 1*b*). Once a subclone develops a full deck of mutations, it will presumably outproliferate those that rely on cooperation. The hypothesis of cooperation among tumor cells implies that a tumor with a full deck of mutations will evolve from a normal cell much faster with the possibility of cooperation, because during the intermediate stage when no cell has yet accumulated a full deck, the tumor can display (fast) malignant growth. This fast growth means that cells that are missing only a sharable resource to have a full deck might be proliferating at a very fast rate, rather than proliferating little or dying, as expected in the traditional view.

Consistency with Known Facts

The hypothesis of cooperation among a heterogeneous population of partially transformed tumor cells is based on sharable resources at the cellular level. This is consistent with evidence that at least three of the hallmarks involve sharable resources: angiogenesis, self-sufficiency of certain growth signals, and tissue invasion and metastasis.

Angiogenesis. The primary mediator of new blood vessel growth within a tumor is VEGF. VEGF, secreted by a tumor cell, recruits the growth of new blood vessels into the area (neoangiogenesis) resulting in additional blood supply (20, 21). This is an example of commensalism; because the products of the blood supply, oxygen and nutrients, themselves diffuse, all of the nearby cells are helped, not just the cell that secreted the VEGF. This type of cooperation has already been demonstrated for cancer cell–stromal cell interactions. For example, cancer cells that express the *ras* oncogene induce down-regulation of the production of the angiogenesis-inhibitory factor thrombospondin −1 by stromal fibroblasts in a paracrine and distance-dependent manner (20), creating a permissive environment for the growth of new blood vessels, resulting in a favorable environment for all nearby cancer cells (as well as the fibroblasts themselves, resulting in desmoplasia).

Self-Sufficiency of Certain Growth Signals. Cancer cells produce several stroma-modulating GFs that are usually associated with wound healing, including VEGF, PDGF, and TGF-β (refs. 9 and 12; Fig. 2). These factors, through commensalism, act in a paracrine fashion, not only to induce stromal reactions for angiogenesis and inflammation, but also to activate stromal cells such as fibroblasts, leading to the secretion of other GFs and proteases (13, 14, 22). Cancer cells use multiple GFs to maintain proliferation that can be obtained in an autocrine or paracrine manner. The concept of mutualism is supported, but not proven, by the heterogeneity of the concomitant expression of GFs and their respective receptors in adjacent cancer cells within a tumor (23, 24). By immunohistochemical/immunofluorescence double-staining techniques of GFs/GF receptors, evidence of three potential paracrine interactions (defined as one adjacent cell expressing the GF and another nearby cell expressing the receptor) was demonstrated for TGF-α–EGF receptor, PDGF-A-PDGFαR, and VEGF-Flt-1 in breast cancer (23, 24). These experiments demonstrate tumor cell heterogeneity of GFs and receptors and suggest that partially transformed clones could share resources and increase the likelihood of each other's proliferation and survival.

Tissue Invasion and Metastasis. Several examples exist of how cancer cells interact with stromal cells to create a more robust tumor microenvironment. Under normal conditions, epithelial cells that have lost contact with their basement membrane receive apoptotic signals of the anoikis type from the invaded tissue and are therefore eliminated. Traditionally, it was thought that the only way to circumvent this was for the cancer cell to develop a mutation that allowed survival with loss of contact inhibition (anchorage-independent growth). At least one factor,

stromelysin-3, is expressed by peritumor fibroblasts and acts as an active partner of cancer cells to reduce their death rate when they invade adjacent connective tissues at the primary tumor site (25). Furthermore, proteases from recruited cancer-associated stromal cells (e.g., fibroblasts, inflammatory cells, and endothelial cells), as well as cancer cells themselves, are known to contribute to pathways critical to neoplastic progression by degrading the extracellular matrix, allowing further cell proliferation, as well as tissue invasion and eventual metastasis (15). This represents another form of cooperation, that of mass action rather than exchange. Mass action cooperation has been documented in other systems in nature, e.g., the production of biofilm by bacteria that protect all of the individual bacteria from a hostile environment (26). Stromal fibroblasts can also be recruited to contribute to tumor formation in other ways. For example, cancer-associated fibroblasts present in invasive human breast carcinomas promote tumor growth, angiogenesis, and cancer cell motility through elevated secretion of the chemokine SDF-1 (27). These examples suggest a paradigm in which different clones and cells within the microenvironment of the tumor are recruited, or evolve, through paracrine mutualism and commensalism interactions to form a complete tumor system.

The above are specific examples of sharable resources, but several points should be noted. First, response to sharable resources may be due to nonshared mechanisms, such as increased GF receptors of a cell that may not benefit other cells in the neighborhood. Second, different mutations may be acquired by different subclones in parallel, rather than sequentially by a single subclone (28–31). This form of cooperation, through the efficiency of specialization, has been clearly demonstrated in economics (32). Third, not all hallmarks of cancer need be shareable. Fourth, the hypothesis of intratumor cooperation does not exclude the development of a single subclone of cells with the full deck of cancer hallmarks. Without question, fully transformed cells do exist. We hypothesize that partially transformed cells can exist together by cooperating and can evolve into cells with the full deck of hallmarks to become malignant.

The possibility of cooperation does not exclude but rather supplements the traditional explanation of cancer as a single subclone being able to achieve all of the necessary hallmarks. Cooperation could actually enhance the emergence of a subclone with a full deck, because cooperation among different subclones in a premalignant lesion could supply a full deck until one of the subclones attains all of the hallmarks on its own. This also applies to cancer stem cell theory (33). Cancer stem cells are also thought to arise through a series of mutations, and cooperation theory can be applied to a population of partially transformed cancer stem cells as they evolve within the stem cell niche/microenvironment.

Cooperation Theory Applied to Tumorigenesis

The delineation of cooperation in tumor–microenvironment interactions has given cancer biologists a window into understanding the changes to the extracellular matrix and normal cells that are induced by their interaction with cancer cells (9, 12–16). This recruitment of normal cells to supply cancer cells with factors that encourage survival has been established in multiple examples, e.g., production of thrombospondin by fibroblasts and the release of TGF-β from bone collagen by osteoclasts. Indeed, the recruitment of normal cells leading to a more robust tumor mass is not surprising, now that it has been documented.

What has not been previously considered is that partially transformed cells may cooperate with each other to form a more robust population of tumor cells. This can be done by recruitment of normal cells, changes to the microenvironment accomplished together or in parallel, recruitment of each other, or sharing paracrine factors (it should be noted that paracrine factors can be either diffusible or shared through matrix connections). The most straightforward example of cooperation between two partially transformed cells is through the production and sharing of two different GFs. In this scenario, the production of a GF A by cell A, which stimulates itself in an autocrine manner, will help the growth of other cells nearby that have the receptor for GF A but are not producing it. If cell A is producing GF A; then, at no additional cost to itself, it helps the other cells in its immediate neighborhood. If cell B acquires a mutation to produce a different extracellular GF that cell A requires, and if the diffusion distances of cell A and cell B overlapped, then some cells would benefit from both factors. In that case, cells A and B would be crossfeeding each other as well as feeding other cells in the area (Fig. 2).

This crossfeeding is a form of cooperation, in that the two subclones are both able to attain a proliferative advantage together that neither has attained on its own. If each cell was able to help the other at no additional cost to itself, the form of cooperation would be by-product mutualism. Cooperation by by-product mutualism is easy to establish and to maintain. In fact, where by-product mutualism is possible, both cells continue without regard to what the other is doing. Unlike cases of cooperation based on reciprocity where each produces a factor required by the other, a "player" engaged in by-product mutualism does not have to make its behavior contingent on the behavior of the another cell line. Several concepts developed in cooperation theory are useful in illuminating specific aspects of the cooperation of cells within tumors. These concepts include nonexcludability, nonrivalry, specialization, free rider, altruism, and reciprocity.

In the case of sharable resources (e.g., GFs), cooperation theory can be applied in a straightforward manner. Indeed, if the sharable resources are nonexcludable and nonrivalous, the evolution of cooperation does not even require contingent strategies. A resource is said to be nonexcludable if one cell cannot prevent another from using the resource. This generally applies to products within their range of diffusion. Nonrivalous means that when one cell uses a resource, it does not limit the amount available to others. The provision of oxygen in angiogenesis is a good example (discussed above). The diffusion of oxygen means that when vascular growth is induced, millions of cells may benefit.

When exchangeable resources are nonexcludable and not rivalous, cooperation can occur in the form of by-product mutualism. This kind of cooperation is based on specialization. It does not suffer from the "free-rider" problem in which only one individual benefits at the cost to another, because what helps others is a costless by-product of what helps oneself. Therefore, by-product mutualism is the easiest form of cooperation to be sustained. It does not require contingent action (unlike altruism based on relatedness or reciprocity).

Based on the hypothesis of cooperation within a heterogeneous population of partially transformed tumor cells, it is possible that the population of cells can be malignant (i.e., can have a full deck of cancer hallmarks), at least transiently during the life of the tumor, even if none of its individual cells are malignant. In that period in time, the whole population of cooperating cells can accomplish more than any of its subclones can accomplish on its own. Malignancy would then exist as an emergent property of the population of partially transformed cells; or, more completely, malignancy may be the emergent property of the population of partially transformed cells, together with normal cells, and the microenvironment (11, 34).

What May Be Explained by the Hypothesis of Cooperation Among Tumor Cells?

Our hypothesis of tumor cell cooperation by sharable resources and capabilities may help provide an explanation of several observations that have been made about solid tumors,

including the following. (*i*) The nonuniform abundance of proteins observed in different regions of tumor tissue visualized by immunohistochemical staining (refs. 23 and 24; not all of the cells have the ability to produce resources the tumor as a whole needs). (*ii*) The heterogeneity of genotypes and phenotypes (refs. 35–38; individual cells provide and use different resources). (*iii*) The heterogeneity of response to cytotoxic drugs (ref. 39; not all of the cells have developed resistance to particular agents). (*iv*) The inefficiency of metastasis (ref. 40; accomplished only by cells with a full deck of cancer hallmarks or by clumps of cooperating partially transformed cells). (*v*) Inefficiencies of cell culture, as demonstrated by the difficulty of establishing cell lines from tumors (loss of cooperating cells within the tumor microenvironment), the necessity to pass some tumor-derived cell lines at high concentrations (requirement for continued cooperation *in vitro*), and the relative low plating efficiency of single cells derived from such lines (accomplishable only by cells with a full deck of hallmarks, or the cancer ''stem cell''; ref. 38).

The hypothesis of cooperation among tumor cells suggests that, in addition to the independent development of a subclone of cancer cells with the full deck of mutations, the accumulation of different mutations in different cells during tumor progression may result in subclones with different abilities and requirements. These subclones may interact synergistically with each other and the other cells of the tumor microenvironment, which is reflected by the fact that, when separated at low cell concentration *in vitro*, they cannot efficiently crossfeed each other (low plating efficiency).

What Is Predicted by the Hypothesis of Cooperation Among Tumor Cells?

The hypothesis of cooperation between partially and fully transformed tumor cells suggests several predictions, which can be tested by observations of tumor tissues *in situ* or experiments with tumor-derived cells *in vitro*.

(*i*) Histological sections of tumors stained for two different GFs by immunohistochemistry are predicted to show nearby cells expressing different GFs.

(*ii*) Adjacent regions of microdissected tumor tissue, when analyzed for gene expression by DNA microarrays, are predicted to express RNAs coding for complementary sharable resources and their concomitant receptors.

(*iii*) Tumor-derived cells grown in culture and diluted to low cell concentration are predicted to reveal cells with different and complementary properties. For instance, some tumors whose cells proliferate *in vitro* at high, but not low, cell concentrations will be found to have subpopulations of cells that proliferate in media with some GFs, and other subpopulations of cells that proliferate with different GFs. The two populations will be found to be able to crossfeed each other; this could be tested by replica plating colonies derived from clumped mixtures of cells compared with colonies derived from single cells.

(*iv*) Cell lines that are known to require two GFs to grow in culture provide a model system to study cooperation *in vitro*. An aliquot of these cells could be transfected with a gene coding for one of the exogenously expressed GFs, and a different aliquot of cells could be transfected with the gene for the other GF. It is predicted that GF independence would be achieved in a mixture of the two cell types.

(*v*) Other assays could compare single cells and clumped mixtures of tumor-derived cells for their ability to carry out *in vitro* transformation phenotypes that have been correlated with *in vivo* malignancy, including motility, invasion, and anchorage-independent growth. For each assay, it is predicted that clumps of mixtures of cells will be able to cooperate and achieve functions that individual cells or single-cell-derived colonies could not achieve.

New Questions

The value of a new hypothesis often derives as much from the new questions it raises as from the accuracy of the predictions it makes. The hypothesis of cooperation among tumor cells suggests the following new questions:

- What is the list of sharable resources and abilities?
- What are the mechanisms by which sharing occurs?
- What are the implications for the expected order of mutations, given that some can be in parallel?
- How can cooperation among partially transformed tumor cells be interrupted to stop, or at least slow, the progression to malignancy?

The theory of cooperation has already provided multiple disciplines, from bacteriology to economics, with valuable insights of how systems evolve that benefit the individuals within them (1–7). The recognition that cancer is the result of a complex interaction of tumor cells with their microenvironment has already led to new therapeutic paradigms (41). The hypothesis of cooperation among tumor cells themselves provides a new framework for therapeutic design as its predictions are tested, and the questions it poses are answered.

We thank Stephanie Forrest and Ross A. Hammond for thoughtful discussions. R.A. was supported by National Science Foundation (NSF) Grant SES-0240852. D.E.A. was supported by NSF Grant IIS-0312953, National Institutes of Health (NIH) Grant U56 CA113004, and New Jersey Commission on Cancer Research Grant 1076-CCR-SO. K.J.P. is an American Cancer Society Clinical Research Professor and is supported by NIH Grants CA69568, CA102872, and CA093900.

1. Dugatkin, L. A. (1997) *Cooperation Among Animals* (Oxford Univ. Press, Oxford, U.K.).
2. Turner, P. E. & Chao, L. (1999) *Nature* **398,** 441–443.
3. Nowak, M. A. & Sigmund, K. (1999) *Nature* **398,** 367–368.
4. Trivers, R. (1985) *Social Evolution* (Benjamin Cummings, Boston, MA).
5. Smith, M. & Price, G. R. (1973) *Nature* **246,** 15–18.
6. Axelrod, R. & Hamilton, W. D. (1981) *Science* **211,** 1390–1396.
7. Hamilton, W. D. (1964) *J. Theor. Biol.* **7,** 1–57.
8. Nowell, P. (1976) *Science* **194,** 23–28.
9. Mueller, M. M. & Fusenig, N. E. (2004) *Nat. Rev. Cancer* **4,** 839–849.
10. Hanahan, D. & Weinberg, R. A. (2000) *Cell* **100,** 57–70.
11. Pienta, K. J. (2005) *Complex Systems Science in BioMedicine* (Kluwer, Dordrecht, The Netherlands).
12. Liotta, L. A. & Kohn, E. C. (2001) *Nature* **411,** 375–379.
13. Klein, C. A. (2003) *Adv. Cancer Res.* **89,** 35–66.
14. Weaver, V. M. & Gilbert, P. (2004) *J. Cell Sci.* **117,** 1287–1290.
15. Tlsty, T. D. (2001) *Semin. Cancer Biol.* **11,** 97–104.
16. Ronnov-Jessen, L., Petersen, O. W., Koteliansky, V. E. & Bissell, M. J. (1995) *J. Clin. Invest.* **95,** 859–873.
17. Vineis, A., Matullo, G. & Manuguerra, M. (2003) *J. Epidemiol. Commun. Health* **57,** 89–95.
18. Tomlinson, I. P. M. (1997) *Eur. J. Cancer* **33,** 1495–1500.
19. Gatenby, R. A. & Vincent, T. L. (2003) *Cancer Res.* **63,** 6212–6220.
20. Kalas, W., Yu, J. L., Milsom, C., Rosenfeld, J., Benezra, R., Bornstein, P. & Rak, J. (2005) *Cancer Res.* **65,** 8878–8886.
21. Hanahan, D. & Folkman, J. (1996) *Cell* **86,** 353–364.
22. Chung, L. W., Baseman, A., Assikis, V. & Zhau, H. E. (2005) *J. Urol.* **173,** 10–20.
23. de Jong, J. S., van Diest, P. J., van der Valk, P. & Baak, J. P. (1998) *J. Pathol.* **184,** 53–57.
24. Royuela, M., Ricote, M., Parsons, M. S., Garcia-Tunon, I., Paniagua, R. & de Miguel, M. P. (2004) *J. Pathol.* **202,** 41–49.
25. Rio, M. C. (2005) *Biochimie* **87,** 299–306.
26. Crespi, B. J. (2001) *Trends Ecol. Evol.* **16,** 178–183.
27. Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V. J., Richardson, A. L. & Weinberg, R. A. (2005) *Cell* **121,** 335–348.
28. Hahn, W. C. & Weinberg, R. A. (2002) *N. Engl. J. Med.* **347,** 1593–1603.
29. Michor, F., Iwasa, Y., Vogelstein, B., Lengauer, C. & Nowak, M. A. (2005) *Semin. Cancer Biol.* **15,** 43–49.
30. Vogelstein, B. & Kinzler, K. W. (1993) *Trends Genet.* **9,** 138–141.

31. Sontag, L. & Axelrod, D. (2005) *J. Theor. Biol.* **232,** 179–189.
32. Smith, A. (1776) *The Wealth of Nations* (W. Strahan and T. Cadwell, London), reprinted (2003) (Bantam Classics, New York, NY).
33. Wicha, M. S., Liu, S. & Dontu, G. (2006) *Cancer Res.* **66,** 1883–1890
34. Vicsek, T. (2002) *Nature* **418,** 131.
35. Shah, R. B., Mehra, R., Chinnaiyan, A. M., Shen, R., Ghosh, D., Zhou, M., Macvicar, G. R., Varambally, S., Harwood, J., Bismar, T. A., *et al.* (2004) *Cancer Res.* **64,** 9209–9216.
36. Miller, B. E., Miller, F. R., Leith, J. & Heppner, G. H. (1980) *Cancer Res.* **40,** 3977–3981.
37. Kuukasjarvi, T., Karhu, R., Tanner, M., Kahkonen, M., Schaffer, A., Nupponen, N., Pennanen, S., Kallioniemi. A., Kallioniemi, O. P. & Isola, J. (1997) *Cancer Res.* **57,** 1597–1604.
38. Von Hoff, D. D., Forseth, B. J., Huong, M., Buchok, J. B. & Lathan, B. (1986) *Cancer Res.* **46,** 4012–4017.
39. Axelrod, D. E., Gusev, Y. & Gamel, J. W. (1997) *Cancer Chemother. Pharmacol.* **39,** 445–451.
40. Micke, P. & Ostman, A. (2005) *Exp. Opin. Ther. Targets* **9,** 1217–1233.