Return of de-differentiation: why cancer is a developmental disease
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Many important advances have been made in the phenotypic and genetic characterization of malignant tumors since the publication of Peter Nowell's seminal article on the origin of cancer, but there has been no consistent effort to incorporate this wealth of knowledge into a general model of carcinogenesis. Current theoretical discussions on cancer are frequently dominated by attempts to categorize genetic alterations and phenotypic characteristics and establish correspondences between them. In this article, I argue, on the basis of recent data as well as "old" observations, that a developmental error leading to the acquisition of a unique cell character (de-differentiation) underlies all phenotypic characteristics of cancer cells and discuss how this notion can be reconciled with Nowell's model of carcinogenesis as a microevolutionary process into an updated theoretical description of cancer.

What is cancer: Nowell's model
What is cancer? An important part of the answer to this fundamental question is embodied in the clonal evolution model of carcinogenesis, summarized by Peter Nowell in 1976 [1]. According to the model, a tumor starts when, through a specific genetic alteration, a single cell acquires a selective advantage over its neighbors. That cell therefore multiplies and gives rise to an abnormal clonal outgrowth. Within this soma-clonal variant, the same process is repeated, generating a more aggressive subclone that takes over the tumor. Multiple rounds of mutation and clonal selection ensure that ever more abnormal and aggressive tumor variants are generated continuously, leading to tumor progression. At the conceptual level, Nowell’s model has three essential elements: Cancer is a microevolutionary process; cancers are clonal; cancer is a genetic disease. The last two have guided a substantial portion of the efforts (and successes) of cancer research in the past two decades, to the point that “cancer is a genetic disease” has become the Central Dogma of cancer research. “Cancer is an evolutionary process,” on the other hand, was a notion comparatively neglected by the cancer community, both in research and in education. Only in the last couple of years, as a result of the growing interest in genetic instability, has the topic regained more visibility. Of course, the number of studies suggested by describing cancer as an evolutionary process is relatively small, whereas “cancer is a genetic disease” points to a whole research program, starting with the identification of the genetic alterations responsible for each step of tumor progression.

Searching for the nature of cancer: genetic and phenotypic analyses
Many genetic alterations will be tumor-type specific, but one would hope they can be grouped in classes, thus helping to uncover general principles about the nature of cancer. This is essentially a “reverse genetics” approach. One tries to identify the players and understand what they do, and, from this information, what is specifically abnormal about the tumor. Another approach is to use “classical” or “forward” genetics: start by determining the phenotypes of cancer cells and tumor masses, deduce what functions are abnormal, and finally identify the genetic abnormalities that cause the phenotypes observed. In each case, the final step serves to validate the starting hypotheses. When using reverse genetics, the predicted effects of a genetic alteration should be observed in a tumor. Conversely, when
following forward genetics, mutations should be found in the predicted genes. In practice, neither approach is strictly followed, but rather a mixture of the two. Since multiple genetic alterations are usually present in a tumor, it is usually not an easy task to demonstrate that a specific alteration causes a defined phenotype. Genetic analyses can frequently demonstrate that mutations in certain genes are essential for tumor development, but the functions of those genes in tumorigenesis are usually “assigned” by the rather biased method of “matching” them to one of a list of “interesting” phenotypes. And forward genetics is even more biased. Because cancers display so many phenotypic abnormalities, even determining what the fundamental phenotypes of tumors are is not a trivial matter. Objectively attributing them to specific cellular functions is extremely hard, and even more so to trace them to specific mutations.

The list of characteristic phenotypic abnormalities of tumors one gets depends therefore on the time and the author [2,3,4•], but several features are frequently mentioned, including the following ones.

1. Tumor cells divide faster than normal, disregarding physiologic mechanisms of growth control. This was probably the first phenotypic abnormality to be considered fundamental, and it is still one of the top choices. It sounds reasonable; after all, cancers do get larger and larger.

2. Levels of apoptosis are reduced. In some tumors, the rate of cell division was found not to be higher than in the corresponding normal tissues. If their cells are not born faster than normal, it was reasoned, their death rate must be lower than normal. Apoptosis regulation is immensely popular and one of the favorite “candidate functions” for tumor suppressor genes.

3. Tumors can regenerate themselves an infinite number of times. In vivo, a single cell is enough to repopulate the tumor; in vitro, tumor cells can be grown forever. The favorite candidate function here is telomerase activity, but despite all the hype, it is highly questionable whether telomerase activation plays any causal role in carcinogenesis.

4. Tumor cells have a capacity to invade and metastasize. Operationally, this is the defining feature of malignancy, and one of the least understood.

5. Angiogenesis is induced. It is now generally accepted that without angiogenesis, tumors would quickly exhaust their blood supply and most likely never become life threatening. Angiogenesis is a rising star, maybe capable of outshining apoptosis.

6. Tumor cells undergo “de-differentiation.” Cancer cells frequently appear less differentiated than their normal counterparts, but whether they actually back-tracked along their normal differentiation route, or simply suffered a differentiation block (and the term de-differentiation is a misnomer) was a matter of intense debate. De-differentiation is a classic concept gone out of favor, and, in my opinion, the ugly duckling of cancer research.

Why cancer should be considered a developmental disease

To explain why I think de-differentiation is so important, I would like to go back to some of the other “essential” features of cancer, starting with invasion and metastasis. To invade and metastasize, tumor cells have to be able to migrate out of the tissue compartment where they originated, enter, survive into and exit the blood stream, set up colonies in other organs, induce normal cells to help them, and so on. This is strikingly reminiscent of a developmental process [5••]. In fact, invasion and metastasis occur at multiple stages during embryonic development (we just name them differently in the developing embryo). The similarity between cancer and embryonic cells has been recognized for a long time. Some childhood and adolescent tumors were even described as being of “embryonic origin.” They were thought to be derived from embryonic remnants, cells that should either have been eliminated or have followed a specific developmental program, but failed to do so owing to some developmental error. Trapped in this “embryonic” state, they kept trying to help form whatever tissue they were meant to, but only managed to create an abnormal and malignant tissue instead. The same can be said about teratocarcinomas, highly malignant tumors that frequently contain a variety of differentiated tissues, including muscle, bone, teeth, and hair. Mouse experiments demonstrated that the same cells can give rise to a teratocarcinoma, or function as normal embryonic stem cells, depending on whether they are injected in blastocysts or adult animals [6,7]. It is pretty clear, in both cases, that the malignancies are the consequence of a wrongful execution of the developmental program of one (or a few) cells and a failure of coordination between the expression of the developmental programs of those cells and the rest of the organism. These tumors should properly be considered developmental diseases. But how can the same conceptual framework be extended to other tumors? The answer is de-differentiation. Although the word development mostly brings to mind images of pattern formation in embryos, development processes extend well beyond the embryonic stage. Lungs, for example, are completely formed only after birth, and breasts after pregnancy [8], whereas the immune system is constantly being rebuilt, and cell fate determination, an integral part of development, is at work throughout the life of an individual. De-differentiation is simply a developmental error that happens also in mature tissues. It is not just a
reversion to a more primitive cell type, however. It is a true error of development, leading to a new, nonstandard, cell type. That cell does indeed have a more primitive character, but also the ability to follow developmental pathways that are not accessible to any normal cell, at least under similar conditions (that is why I prefer the term dysdifferentiation), and it is this singularity that makes it an abnormal, malignant cell. De(dys)differentiation is the fundamental feature of cancer cells. All the other properties mentioned should be considered secondary to this developmental aberration. Increased cell division, decreased cell death, unlimited capacity for self-regeneration, cell migration and tissue remodeling, angiogenesis induction (which occurs many times during embryogenesis, and in fact is only one of many examples of cross-talk between different cell types leading to the formation of new structures), increased expression of “fetal” messages, all are natural consequences of the acquisition of a more primitive “stem-cell like,” or “embryonic” character. The ugly duckling has turned into a swan.

Considering what we know about the role of differentiation blocks in hematologic malignancies [9–12], a description of these cancers as developmental diseases seems perfectly appropriate; but how about the more common carcinomas? Some facts about breast cancer incidence (eg, positive correlation with length of estrogen exposure, negative with early parity) and the special nature of breast development (eg, long periods of quiescence, completion only with lactation) strongly suggest that breast cancer is also due to developmental abnormalities. And this point of view is supported by experimental results demonstrating that breast cancer oncogenes and tumor suppressor genes play important roles in normal breast development [13•,14–16]. But an even stronger case can be made for colorectal cancer, the most thoroughly characterized of all common tumors. Early hints that colon cancer is due to a developmental defect can actually be found in one of the first studies to show that inactivation of the tumor suppressor gene APC is the initiating event in the majority of colon tumors [17–21]. Molecular analyses of microscopic lesions of the colon showed that APC mutations cause dysplasia—and therefore a change in cell character—and that dysplasia, not simply hyperplasia, is necessary for colon cancer initiation [22,23]. The next important piece of evidence came a few years later, in the form of a detailed morphogenetic analysis of the intestinal tumors caused by APC inactivation in mice [24]. Tumors were formed by cells in the proliferative zone of the crypt that, instead of continuing their differentiation-coupled upward movement along the crypt-villus axis, retained crypt-cell-like features and actually continued to proliferate into the villus, as if continuously trying to re-form a crypt. More recently (and after previous work pointing to the usual suspects apoptosis [25] and cell migration [26,27]), it was determined that the major biochemical function of APC is to downregulate β-catenin levels and thus the level of transcription by β-cat/TCF4 complexes in the colonic epithelium (and other tissues) [28•,29,30,31•,32]. What this means at the cellular level can be seen from the phenotype of TCF4 knockout mice. The animals die shortly after birth due to an almost complete absence of stem cells in the gut, demonstrating that β-cat/TCF4 signaling is necessary for the maintenance of a stem-cell population [33]. This implies that the main function of APC, at least in the mouse, is to control the stem cell to daughter cell transition, that is, to determine cell fate, in the intestinal epithelium. Colon cancer is also a developmental disease.

Toward an updated theoretical description of cancer

I just argued, primarily on the basis of a phenotypic analysis, that cancer is a developmental disease. On the other hand, for the past two decades, cancer research has been dominated by genetics and the view that cancer is a genetic disease. Is there a way to reconcile these two views into an updated theoretical description of cancer? Let me start by pointing out that, even from the point of view of Nowell’s model, the current focus on genetics should be considered excessive. All the excitement about cancer genetics has obscured the central tenet of Nowell’s model, as true today as it was 25 years ago, that carcinogenesis is a microevolutionary process. It is evolution that drives tumorigenesis; genetic alterations are only one of the “ingredients” of evolution. Next, let us consider what the result of that evolutionary process is. Because of the somatic mutations occurring during their development, cancer cells’ genomes are different from those of normal cells. By itself, this is not particularly important. The “background” rate of somatic mutations is high enough to ensure that soma-clonal variation is rather common in multicellular organisms with large genomes. But the normal variants are still responsive to physiologic regulatory mechanisms, and keep working toward the general goal of helping to ensure the survival of the germ-line. Malignant soma-clonal variants—ie, cancer—on the other hand, have a germ cell character themselves and evolve independently of, and competing against, the organism in which they arose. In other words, the evolutionary process of carcinogenesis gives rise to new (and parasitic) organisms. Cancers resemble embryos because they both are developing organisms, resorting to the same mechanisms “invented” by evolution to overcome similar problems. Angiogenesis, for example, is evolution’s response to a nutrient and oxygen limitation, a tool that both tumors and embryos use to overcome a similar need. But cancers, being distinct organisms evolving along distinct paths, are not constrained by the same
limitations that ensure the proper development of the organisms from which they originated. Starting (for the most part) with genetic alterations, their cells have access to and acquire a broad range of cell fates that, because of their deleterious nature, are forbidden to the normal cell. Now one can see how the descriptions of cancer derived from genetics and phenotypic analysis can be reconciled. Genetic alterations in cancer are likely to affect developmental processes, because in microevolution, as in (macro)evolution, new organisms can be efficiently generated by modulating those processes. By changing a multicellular organism’s developmental programs, evolution can easily build a new organism with an entirely new (parasitic) character out of the cells of the original one.

This attempt to put forward an updated theoretical description of cancer is not solely a speculative exercise. Theoretical models, whether explicitly formulated or not, influence decisions on the lines of research to be pursued, as well as the interpretation of results. According to the model I outlined, we should probably redirect most of the effort put into the study of apoptosis to the elucidation of the mechanisms of organ and tissue formation and cell-fate determination. And maybe we should try to find cancer drugs by developing assays to identify compounds that can induce differentiation [11,12,34]. And the role of BRCA1 in cancer might have absolutely nothing to do with genetic instability. More important still, changing theoretical frameworks can lead us to consider new possibilities. If we think of cancers as developing organisms, for example, it is only natural to expect them to be composed of different cell types. Under this light, Judah Folkman’s “old” remark that tumors are complex tissues, comprising both normal and neoplastic “compartments” should be considered one of the most important conceptual advances in cancer research; but a whole range of other possibilities, not all of which are completely speculative, can be envisaged. Some cancers might be initiated by cells with normal genomes, as in certain polyposis syndromes in which precursor lesions to the carcinomas (epithelial-cell derived) are caused by genetic alterations in stromal cells [35]. Some cancers might be clonal but be composed of different neoplastic cell types [36•,37•], all cooperating to improve the overall fitness of the organism. In other cases, the component neoplastic cells necessary to make the tumor survive might even come from different clones, with different genomes (indeed polymonality has been described in breast cancers [38] and observed to be partially preserved in tumor metastasis [39]) in some cases derived from the original “founder” cell, in others from independently derived mutant cells. In one aspect, Nowell’s model might be changed significantly; cancer organisms are the result of an evolutionary process, which reorients developmental programs, but they might not always be clonal after all.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

Of special interest
** Of outstanding interest

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I found this article and the one by Aigner et al. [37] almost 6 months after writing what I thought was a theoretical speculation. I was pretty excited to find out that tumors like these might indeed exist. However, it is not clear whether the two parts of the tumor are really cooperating, not just co-localizing and, even if they are, whether the split is due to an additional genetic alteration or not.

