Perspectives

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Rudolph Virchow and the Genetic Basis of Somatic Ecology

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"CANCER is a genetic disease, arising from an accumulation of mutations that promote clonal selection of cells with increasingly aggressive behavior." This single terse statement from Fearon (1997, p. 1043) is a paradigm that states the fundamentals of our present understanding of the origin and nature of cancer. The work leading to this understanding of the many pathological conditions called cancer that afflict humans, and many other animals, has occupied the full energies and efforts of uncountable numbers of biologists, biochemists, and physicians going back at least 140 years, starting with the observations of Rudolph Virchow (1821–1902). Virchow was a polymath of the same rank as his senior contemporary, Goethe: a physician, pathologist, cell biologist, ethnologist, archaeologist, anthropologist, teacher, and statesman.

In 1858 Virchow gave a series of 20 lectures to a group of physicians at the Institute of Pathology in Berlin. These were published in the same year under the title *Die Cellularpathologie in ihrer Begrundung auf physiologische und pathologische Gewebelehre* (Virchow 1858). In these lectures he summarized many of the ideas arising from his 12 years of previous experience as a practicing pathologist studying the microscopic anatomy of tissues with special attention to those deviating from the healthy condition. He came to the conclusion that the pathological histological conditions he observed resulted in modifications of the interrelationships of body somatic cells, leading to changes that I shall call somatic ecology. Virchow is now generally best known not for this observation, but for the aphorism he stated in Lecture II of this series: "*omnis cellula e cellula*" (all cells come from cells).

It was not always obvious that cells come only from cells. Earlier in the nineteenth century Schleiden (1838) and Schwann (1839), among others, made it apparent that all plants and animals are constructed of cells, and this came to be known as the cell theory, which was an important step forward in the understanding of the structure of organisms, but was sadly deficient at the time as an explanation of where cells came from. It was generally accepted in the 1850s that new cells were created from a formless, fluid exudate within existing cells, the blastema. This was nothing short of spontaneous generation, but was nonetheless a theory adhered to by some of the most distinguished medical researchers and biologists of the time. The idea that new cells arise from the division of preexisting cells was not original with Virchow, however; he regularly referred to the observations of his friend Robert Remak (1852), an embryologist, who as early as 1841 observed cell division in frog blood cells. But through Virchow's growing influence, amplified by the publication of *Cellular Pathology* (1863), the concept *omnis cellula e cellula* began to be generally accepted and led to the realization that life under existing conditions never arises *de novo*. Instead, each cell belongs to an infinite pedigree, an unbroken series of cell divisions stretching backward from our own time throughout the past history of life. As Wilson (1925) has rightly pointed out: "This terse phrase embodies one of the most important generalizations of modern science" (p. 114). From it Virchow was apparently the first to deduce the origin of cancer.

A close reading of the English version of *Cellular Pathology* makes this quite clear. This version, first published in 1863 as *Cellular Pathology as Based upon Physiological and Pathological Histology*, was a translation from the second German edition by Frank Chance, a British physician fluent in German and a close friend of Virchow's. The translation process was closely monitored by Virchow, who was fluent in English. We therefore can assume with some confidence that the English version truly represents Virchow's thoughts. My comments here are based on a reading of the Dover edition of 1971, an unabridged and unaltered republication of the English translation.

The first three lectures of *Cellular Pathology* contain the essence of Virchow's thoughts relative to the cellular origin of cancer. First, he makes it clear on page 40 of Lecture I that "*Every animal presents itself as the sum of vital unities* [this and all subsequent italics are Virchow's], every one of which manifests all the characteris-

tics of life. . . . Hence it follows that the structural composition of a body of considerable size, a so-called individual, always represents a kind of social arrangement of parts, an arrangement of a social kind, in which a number of individual existences are mutually dependent, but in such a way, that every element has its own special action, and, even though it derives its stimulus to activity from other parts, yet alone effects the actual performance of its duties." The vital unities referred to are the cells: ". . . the cell is really the ultimate morphological unit in which there is any manifestation of life" (p. 29).

Lecture III bears the title "Physiological and Pathological Tissues," and in it Virchow comes to grips with the problem of the pathological tissues that he also calls neoplasms and makes the statement that ". . . every pathological structure has a physiological prototype, and that no form of morbid growth arises which cannot in its elements be traced back to some model which had previously maintained an independent state in the economy" (p. 88). The physiological prototype is the healthy or normal state as opposed to the diseased state, and ". . . a physiological type can be found for every pathological formation, and it is just as possible to discover such types for the elements of cancer. . ." (p. 91). When it comes to the discussion of the transition from the healthy to the neoplastic state, Virchow is at a loss for words, for he is way ahead of his time, and new words such as mutation and clone had yet to be coined. But since he holds consistently to the doctrine that cells come only from cells, it is difficult to avoid the proposition that he is thinking in terms of what we now call somatic mutation. For example, this statement is found on page 99: "In the place of the law of continuity, therefore, we must place something else. And here, I think, the doctrine which has the strongest claims to our attention is that of *histological substitution*." And on page 100 we read: "In diseased conditions *pathological substitutions* occur, in which a given tissue is replaced by another; but even when this new tissue is produced from the previously existing one, the new formation may deviate more or less from the original type. Therefore there is a great chasm between physiological and pathological substitutions, or at least, between the physiological and certain forms of the pathological ones."

Just as Charles Darwin (1859) advanced the theory of common descent accompanied in successive generations by gradual modifications leading to the eventual formation of new species, Virchow at about the same time advanced the theory that abnormal changes in the cells of the body, all derived by common descent from a germ cell, could lead (or evolve?) to a diseased condition such as cancer. The difference between the two versions lies in changes in germ-line cells *vs.* those in the somatic line as later distinguished by Weismann (1892). That there is indeed a somatic evolution of cancer has been well documented, for example, by Bodmer and Tomlinson (1996) and Bodmer (1997) and can now be accepted as established fact.

Virchow continued to speculate for the rest of his life about his understanding of cancers, and other "pathological substitutions," identified histologically as lines of cells with, in his words, "bad behavior." He gave many lectures at home and abroad in which he advanced the view of human diseases as being the result of "civil war between cells." He thought that Pasteur's germ theory of disease was inadequate for explaining all disease. He believed that changes in the "economy" (ecological conditions?) within the body caused by these cellular substitutions or transformations were more important. However, much of Virchow's own activity after about 1870 was given over to his many other interests, including politics. In the 1890s a fellow physician, von Hansemann, taking his lead from Virchow, advanced a hypothesis he called *anaplasie*, in which he touched upon the possibility of somatic mutation (without actually using the term mutation) leading to cancer (von Hansemann 1892). Later Whitman (1919), in a critical review of von Hansemann's speculations, posited that he was in fact stating that cancer was the result of somatic mutation. Boveri speculated in 1914 that cancer was associated with chromosomal abnormalities, but provided no solid evidence. Experimental evidence for the link between somatic mutation and cancer in mice was provided by Tyzzer as early as 1916.

Much of this speculation, even though supported by evidence from mice, was ignored by most oncologists until prominent geneticists such as Burdette (1955) and Schultz (1959) again began to emphasize the role of somatic mutation leading to neoplastic growth. In Schultz's review he pointed out that, although the methods for the study of mutations in germ cells were not applicable to the studies with somatic cells, it was apparent that they regularly occurred in plants such as maize. Meristematic cells of plants frequently form easily recognized, phenotypically mutant sectors, and the genotypic changes tested in the germ line show Mendelian patterns of inheritance.

But somatic mutation in animals was more difficult to study. As early as 1941, Demerec did demonstrate that miniature wing genes (*m*) in *Drosophila virilis* mutated either in the germ line or in somatic cells. Other examples of somatic mutation were found subsequently in *D. melanogaster*—such as the position effects expressed in somatic cells when a gene has been translocated from its normal position in euchromatin to an abnormal one in juxtaposition to heterochromatin (Lewis 1950). Techniques available for detecting somatic changes in Drosophila were not available for doing the same in mammals, but it was logical to assume that if they occurred in insects they also did so in mammals. To a geneticist like Schultz, the reasonable explanation for the incidence of cancer being higher in some families than others but without a consistent pattern of Mende-

lian inheritance was that either somatic mutation or polygenic determination was involved in the origin of clones of cancerous cells. But he thought that somatic mutation was the most probable cause.

The advent of cell culture beginning in the 1950s, leading eventually to the development of somatic cell hybrids and somatic cell genetics, provided the needed techniques for studying somatic cell mutation and identifying genetic polymorphisms leading to the discovery of new genes and the parasexual mapping of genes in mammalian genomes. Tools with which to deal with cell populations and even single cells became available. Linder and Gartler (1965) took advantage of the newly discovered finding by Lyon (1961) that in the eutherian mammals only one of the two female X chromosomes is expressed in each cell so that the translated products in heterozygotes for the two alleles of a particular gene will be expressed hemizygously in equal numbers in a population of cells. They then showed that only one of the two alleles, A or B, of the sex-linked gene for glucose-6-phosphate dehydrogenase (*G6PD*) was expressed in the benign leiomyomas of human female AB heterozygotes. This was consistent with the concept of clones of cancer cells having their origin from single cells and gave support to the hypothesis that cancer arose by somatic mutation. It should be recognized, however, that at least some cancers are known to have a polyclonal phase (Novelli *et al.* 1996; Merritt *et al.* 1997), but that cancers become clonal is now well established.

At least 99% of the incidences of solid tumor cancers appear to be the result of somatic genetic alterations that initiate the production of aggressive neoplastic cell lines (Fearon 1997). There is a class of "cancer genes" called tumor suppressor genes (TSG), or antioncogenes, the recessive mutant alleles of which can cause cancer when inherited through the germ line. Since the frequencies of nonfunctional mutant TSG^- alleles in the germ lines, and in homozygous TSG^+/TSG^+ somatic cells resulting from somatic mutation, are expected to be extremely low, essentially all occurrences will be in TSG^+/TSG^- heterozygotes. Therefore, the only significant frequency of cancer clone initiation can be by loss of the normal TSG^+ allele in the soma by a process generally referred to as loss of heterozygosity (LOH). This can and does happen by a variety of LOH mechanisms, such as mutation of the heterozygous wild-type TSG^+ to an inactive allele, its loss by deletion or nondisjunction, or the achievement of homozygosity of the inactive mutant allele by mitotic crossing over (Tischfield 1997).

Knudson (1971, 1993) made an important breakthrough in the understanding of the origin of most cancers when he showed that the inherited occurrences of retinoblastoma in one eye only (the so-called unilateral cases) in the human population occur later in life than those in which the dominant bilateral condition is inherited with tumors in both eyes. Semilogarithmic plots of the fraction of cases of both kinds not yet diagnosed at different ages ranging from 1 to 50 months clearly revealed that the time required for the appearance of tumors of the bilateral type follows a simple one-hit curve, while unilateral cases follow a two-hit curve. This led to the general acceptance of what is now known as the two-hit hypothesis: oncogenesis generally requires two mutations, one germinal and one somatic. The germinal mutant allele will nearly always be in a heterozygous condition with an active normal dominant allele in every cell of the body, and only LOH leading to loss of the normal entity can create a neoplastic cell clone. Of course, two somatic mutations occurring in a cell line to produce a homozygous mutant neoplastic clone from a homozygous normal TSG^+ are also possible, but this should be much rarer than a single mutation in a heterozygote.

Although the two-hit process involving the inheritance of a mutant TSG followed by a somatic LOH can be taken as a model for the origin and progress of a cancer, it gives only a partial insight into the progression of the disease after the initial somatic event(s) occurs. The evolution of the process in the soma of an individual carrying a single familial mutant TSG may involve the initiation of not just one but many mutant clones by many somatic "hits" resulting in LOH (Bodmer 1997). Also, many genes other than the TSG may interact and modify the initiation and evolution of the polyclonal neoplasm, as shown by Dove *et al.* (1998) using the progress of tumor formation in the intestinal epithelium of mice heterozygous for a mutant inactive allele of the adenomatous polyposis coli (*Apc*) gene. Mouse *Apc* is homologous to the human *APC*, an important TSG, the mutant forms of which are involved in the initiation of intestinal cancers in humans.

Virchow had a wide perspective on the problem of human disease. He focused on events that he conceived of as leading to evolutionary changes in a restricted region of a single individual animal, resulting not only in diseased conditions such as cancer, but in many other abnormal conditions he recognized as a pathologist. He was right, for we are now realizing that the occurrence of many diseases other than cancer may be following the two-hit rule (Qian and Germino 1997). These, like most cancers, are not inherited with a Mendelian pattern, but do tend to run in families in an erratic manner. One example among many is the human developmental defect, holoprosencephaly (HPE), which causes a variety of forms of abnormal development of the forebrain and midface. Some cases are nearly normal and others severe enough to be incompatible with postnatal life. The human hedgehog gene, Sonic Hedgehog (*SHH*), has been identified with *HPE3* (Holoprosencephaly type 3), a gene known to cause HPE, by Roessler *et al.* (1996), who have also identified the structure of some of the products encoded by the mutant alleles of this

gene. Bearers of these mutant alleles heterozygous with the normal allele do not show HPE, but deletions of the chromosome 7 region, including the *SHH* region, are strongly correlated with HPE (Roessler *et al.* 1997). Hence, the expression of the HPE phenotype may well be the result of LOH and a somatic event.

It is becoming increasingly clear that somatic mutations and related somatic events must now be considered as possibly playing a major role in causing diseases other than cancer, including those that become manifest only later in life, such as Alzheimer's disease and the various forms of arthritis, as well as the many other different rare forms of pathological conditions that can occur at any stage starting in the embryonic period. The accumulation of somatic mutations as we age may be an important factor in the aging process. What Virchow called the changes in the "economy" of the body we can now call changes in "somatic ecology," leading to imbalances that he called the "war between the cells" as a result of genetic changes occurring in the soma leading, in his words, to changes in the "social arrangement of parts," or what we now can refer to as loss of homeostasis. It is not too far fetched to posit that Virchow's ideas about the body's economy relate to the concept of homeostasis. In this he was in accord with an eminent contemporary, Claude Bernard (1813–1878), a prominent father of scientific medicine. Bernard's research as a physiologist convinced him that the maintenance of an internal environment (*milieu interieur*) in which the function of each part of the body was in harmony with every other part was the condition of a healthy state of life (Bernard 1865).

All things considered, it is reasonable to assume that one major factor disturbing the maintenance of homeostasis, *i.e.*, a healthy state, is somatic mutation.

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