INSTRUCTOR'S PERSPECTIVE
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When I was the age of the students in this class circa 1965 my instructors and senior colleagues were pretty sure that the discovery of DNA and its code for all the proteins of life would soon permit understanding of how afflictions like cancer occurred. One leading molecular biologist even wrote to then President Nixon and told him that were he provided with sufficient funding he would discover the causes of cancer in five years. Cancer researchers as a group were similarly optimistic and their testimonies translated into greatly increased federal research funding and the 1972 declaration of the "War on Cancer".

Theodor Boveri had in the late 1890s performed microscopic examination of human chromosomes during metaphase in normal tissue, localized tumors and their derived metastases. He found that aberrant chromosomal forms that were common to the parent tumors and all metastases but that new aberrant chromosomes appeared in metastases that were not found in the parental tumor and were not shared among all metastases. From these data he inferred that cancers arose from a single parent cell but this cell lineage accumulated genetic errors visible as aberrant chromosomal shapes as the tumor grew and created metastases. By the time I was an undergraduate the use x-linked markers in heterozygous females had appeared to confirm the monoclonality of tumors and extended it to atherosclerotic plaque that underlies most forms of vascular disease.

Added to this common understanding were the discoveries by oncovirologists that expression of certain genes (oncogenes) carried by viruses induced tumors in birds, cats and rodents and that these genes had cognate forms (proto-oncogenes) in those animals and humans. When it was found that these genes could be found spontaneously mutated form in these animals and in some tumors induced by treatment with chemicals or radiation the concept of cancer risk from environmental radiation and chemicals acting as mutagens gained wide credence. This mutagens = cancer risk was a plausible hypothesis. The earliest work in Drosophila genetics and then microbial genetics had shown that genetic change could be induced by ionizing irradiation and the existence of the "proto-oncogene" in the human genome and in mutated form in tumors seemed to strongly support the hypothesis.

Consistent with these ideas were the arguments based on a model of carcinogenesis devised by Nordling and extended by Armitage and Doll in the 1950s that turned Boveri's inferences into a quantitative model first of simple accumulation of genetic changes in a cell lineage. Soon thereafter Armitage and Doll created a model that also accounted for the fact that human cancers were preceded, often for many years, by clinically apparent but nonmalignant lesions such as polyps in the colon. Using observations of several forms of familial cancers with pediatric or young adult onset, Knudson inferred that the events were required to begin or "initiate" growth of these precancerous colonies were the losses by mutation of both copies of certain "tumor suppressor genes".

Epidemiologists added their observations showing the beginnings of what was to be a five fold increase of age-specific cancer mortality rates in the mid 20th century that accorded with ideas that the simultaneous growth of the chemical industries and introduction of thousands of new chemicals into the human environment was the dominant cause of these increases. By the early 1950s the epidemiologic evidence that cigarette smoking caused lung cancers was solid on statistical grounds and the premise that the chemical mutagens found in cigarette smoke must have caused the cancers by causing mutations.

In the following years many tumor suppressor genes and proto-oncogenes were associated with human cancer pathways because significant fractions of human tumors were found to carry missing mutant forms of these genes in samples taken from tumors.

The lethal tumors that kill the vast preponderance of cancer patients do so after age fifty with certain marked exceptions such as numerically early forms of breast cancer and nervous system cancers.

Scientists were satisfied with these ideas. Large fractions of cancer and mutation research investments were spent detailing the mechanisms of how environmental mutagens act in non-human systems and, unbelievably now, the scientific world lent its intellectual capital to the proposition that the environmental cancer risks could be discovered by testing individual chemicals for their ability to induce mutations in the bacterium S. typhimurium. Your professor got caught by this series of assumptions, too, and spent some twenty years of his scientific life developing means to test chemicals as mutagens in human cells and searching with environmental engineers and analytical chemists for the chemicals that could mutate human cells in air, food, water and pharmaceuticals.

But I also had the advantage of natural agnosticism and the mentorship Charles Heidelberger at the McArdle Laboratory for Cancer Research who pointed out in 1971, "We now know what causes cancer! Viruses, chemicals and irradiation or something else." I also had the advantage of Salvador Luria's moving 1965 testimony in 7.21 as to his motivation to design and use the "fluctuation test" as a means to discover if bacteria mutated in response to changed environmental conditions or whether changed environmental conditions selected for previously existing mutants that arose spontaneously in the bacterial cultures. "Sal" was a Jewish-Italian refugee from fascist Europe. He spoke of a strong fellow feeling for the leading Soviet geneticists and their students who in the late 1930s and early 1940s were summarily executed for objecting to the contention of the botanical geneticist Lysenko that he had induced the genetic ability of wheat seedlings to grow in cold weather by forcing them to sprout in a cold room. Josef Stalin liked the idea that you could change genetic by environment and planned to do it to humans with the instruments of the totalitarian state. His response to the objections of world-leading Soviet

geneticists, especially in what was then Leningrad was summary execution. My wife's grandmother was dragged from her home in front of her six and four year old daughters and shot in 1941. Ideas have consequences.

Luria's ideas about environmental selection stuck with me and I stayed home for a sabbatical in 1983 to just think out how one could perform assays for genetic change in the human tissues from which common cancers arose. Seymour Benzer had found in 1958 with bacterial viruses that different mutagens produced nonrandom and reproducible quantitative patterns of point mutations or "mutational spectra" along the gene sequences studied. There was also a mutational spectrum for spontaneous mutations, those that arose when viruses simply multyiplied without any added mutagens. I determined to find and or devise technology to observe point mutational spectra in human tissues and use them if possible to discover the causes of point mutations in humans. In late 1983 Lenny Lerman described the basic enabling technology that eventually made such studies possible, denaturing gradient gel electrophoresis, which in its present variant form using controlled temperature capillary electrophoresis, allows measurement of point mutations in human tissue DNA samples at the levels art which they occur in human tissues. Lerman immediately hosted my student as his guest at the Albany Medical College and in the early 1990s with the collaboration of Barry Karger at Northeastern who invented capillary gel electrophoresis universally used for DNA sequencing developed the technology that today permits mutational spectrometry in tissues and large pools of DNA samples. Konstantin Khrapko, now a professor at Harvard Medical School led the developmental effort in my lab. An independent means to measure particular point mutations based on allele-specific PCR was developed in my lab at the same time by Rita Cha who now heads her own lab in Great Britain's National Institute for Medical Research.

With these tools to measure point mutations we found that human cells in culture accumulated the same set of mitochondrial point mutations as found in multiple tissues and their derived tumors. Remarkably many years of cigarette smoking had no effect on the number or kind of mitochondrial point mutations found. More recently nearly all of these mutations have been found by primary errors of the only known human mitochondrial DNA polymerase gamma copying DNA in a test tube. Some of the mutations were ascribable to copying past the natural deamination of cytosine from DNA and the rest were primary errors of the polymerase copying undamaged DNA. The idea that mitochondrial mutations arose primarily from exposure to environmental mutagens was no longer supportable.

Recently these observations were extended in two independent Ph.D. theses to nuclear genes. In sequences of the APC gene mutations of which appear to initiate somewhat more than half of human colon tumors, a mutational spectra was constructed by adding together the results from hundreds of colon tumors bearing APC mutations. Of the mutations found in the APC sequences studied more than half were created when DNA was copied in a test tube by nuclear DNA polymerase beta. Further, studies, completed in 2004, of five point mutations in three nuclear genes in the bronchial epithelium of six smokers and nine nonsmokers found large numbers of mutations in nboth groups but no differences between smokers and nonsmokers.

These observations and ideas appeared in the July 2003 issue of Nature Genetics under the title, *"Do environmental mutagens induce oncomutations in people?"* The silence following this publication has been deafening.

Such phenomena of science notwithstanding it seemed clear by 1988 that the mutation assays under development would succeed in human tissues. I thus turned aside from active laboratory work to explore the epidemiology of cancer with the goal of gleaning from that field some important hypotheses about the origins of that could be tested by accurate tissue mutation assays. What I found was not very helpful. Neither the National Cancer Institute nor the American Cancer society had bothered to collect and organize the national data on mortality from different forms of cancer that had been recorded by the U.S. Census Bureau (1890-1935) and the U.S. Public Health Service in a way that could be analyzed by interested scientists. It fell to my graduate student, Pablo Herrero-Jimenez, to personally realize the value of such a task and, unasked by me, to perform it as part of his Ph.D. thesis. These are the principal data of http://epidemiology.mit.edu used in this course that he gleaned by uncounted days and nights in a basement of one of Harvard's libraries. With the data so organized it became easy to recognize that the rise in cancer rate for many cancers that were seen to increase in the 20th century had in fact begun these increases with the decline in agrarian life that began with the birth cohorts of vthe 1830s for European Americans, the 1860s for African Americans and the 1880s for Japanese.

Another curious related scientific phenomenon was that groups developing models of cancer risk in the population had occasionally recognized the possible need, but never formalized expressions accounting for the possibility, that only discrete fractions of the population might be at risk of particular forms of cancer as a result of environmental or genetic risk factors or both. With plenty of help from Pablo Herrero-Jimenez and Stephan Morgenthaler and support for a purely thinking sabbatical from the Swedish Cancer Society, I finally hacked out an initial model and Pabl wrote the first computer program to permit using raw public health data to analyze the changes in cancer rates for birth cohorts as they aged from zero to 104 years. The first programs could handle fewer than 10^8 computations per day and led us to some injudicious conclusions in which local minima were mistaken for explicit solutions. Later programs placed on faster computers such as the MacIntosh G5 were created based on Morgenthaler's Fortran program transported into Java by two successive M.E. candidate, David Hensle and John Kogel into forms such as CancerFit3_7.jar that can be easily used by non-hacker cancer researchers and BE.102 students.
With regard to inherited risks for common diseases like cancers, I was amazed when I found out that reputable scientists at MIT and around the world were seriously contemplating using the method of linkage disequilibrium analysis. This method depends critically on monoallelic risk in the family population studied. "Brought up" in mutational spectrometry I knew that virtually all genetic risk was decidedly multiallelic as one may verify by reference to http://www.hgmd.org. For common disease risks one should expect relatively common genetic variants and if these are represented by several different mutant alleles in the same gene, they will confound any attempt to locate

the carrier gene either in studies of extended families or scans of the general population of afflicted and unafflicted persons. To pursue the search for the genes encoding risk for common disease I founded a company Peoples Genetics, Inc that has been purchased by Beckman Coulter, Inc and with whom I now work to provide scientists with instrumentation suitable for mutational spectrometry in large human populations.

Most recently my scientific perspective has been extended by the discovery by my colleague and wife, Elena Gostjeva, that the cells that appear to drive the growth and differentiation of human embryos and tumors are a curious form dubbed “metakaryotes” in which both symmetrical and asymmetrical nuclear divisions occur without general chromosome condensation. One interpretation we share based on her histologic observations and our analyses of lifetime cancer rates, observations of mutations in tissues, clinical and human genetics is that preneoplasia appears to be simply a clonal continuation of juvenile growth in adult tissues from which one “stem” cell transforms and tries to create an embryonic organ with usually fatal consequences. We think that environmental changes accompanying urbanization somehow interacted with a relatively small number of genes that accumulated non-deleterious gene-altering or inactivating mutations throughout the genetic life of our present human species. Just what these environmental factors do is still a mystery. Among plausible possibilities are unrecognized infections or other inducers of tissue inflammation. This idea that tumors arose from embryonic cells in adult tissues was actually put forward pretty clearly by Cohnheim in 1875 in Berlin. *Plus ca change, plus ca meme chose.*

It seems to me that no matter which field I have blundered into, genetic toxicology, epidemiology, population genetics, cancer research and, led by Lena, embryology and stem cell research, I have found that things were not as they appeared or generally assumed. What has amazed me is the resistance of scientists to recognizing and testing important assumptions despite unprecedented public funding for scientists since WWII. But young people who hear the call of science need not be discouraged at all. The bleating of the mediocre or the shrill calls of leading lemmings mean little when a beautiful new idea occurs to you alone. If you persevere, these ideas will come.

This little course is called “macroepidemiology” for lack of a sufficiently comprehensive term for its contents. It is offered for those students who accept the responsibility of self-education. They may benefit from the experience of carefully analyzing what is known about a common disease in the company of other students with shared interests and an instructor for whom such analyses are a self-required responsibility.