Chapter 15. The Oncogene Discovery Story 220723aw3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

Kurt W. Kohn, MD, PhD
Scientist Emeritus
Laboratory of Molecular Pharmacology
Developmental Therapeutics Branch
National Cancer Institute
Bethesda, Maryland
kohnk@nih.gov

CHAPTER 15

The Oncogene Discovery Story.

Introduction

The previous chapter was about the *ABL* oncogene, one of the first cancer genes to be discovered. The oncogene discovery story however converged from several different directions. Pathologists had puzzled about the origin of cancer for more than a century -- until an answer emerged from out of the genetic shadows. There were genes known to predispose to cancers in families – yes, but how did they work? An answer to that old complicated and controversial question came from a remarkable discovery – the discovery of "oncogenes", literally "cancer genes," genes associated with cancer causation.

As in the case of *ABL*, an oncogene was often found to be a mutated version of a normal gene, a "proto-oncogene," that had become overactive and consequently pushed the cell to multiply without control. In the previous chapter, we saw an example in the *ABL* gene that was driven to become overactive by a gene from another chromosome, the gene coming to lie next to the ABL gene as a consequence of a recombination event between the two chromosomes.

Another way that cancer may be driven was found to be by inhibition of a "tumor suppressor" gene that normally held cell division in check. The most famous tumor suppressor was *TP53*, whose story will be the subject of a later chapter. The full story of *RAS* oncogenes, one of the most important class of oncogenes, will be told in Chapter 18. Here I will begin the story with how the concept of "oncogene" actually emerged in the context of the first discovered oncogene, *RAS*.

Here is an overview of the various ways oncogenes can arise, as revealed by long and weary studies: A normal gene, a proto-oncogene, can become an oncogene by way of (1) a mutation in the gene; (2) reduced methylation of the gene's promoter region (methylation normally suppresses the gene's activity); (3) increase in the number of copies (amplification) of the gene in the cell, due to an increase in the number of chromosomes, or in chromosome sections (as in homogeneously staining regions, Figure 5.7 in Chapter 5), that have many copies of the gene; or (4) a recombination event where pieces of two different chromosomes become stuck together in such a manner that an activator region in one chromosome piece abnormally activates a proto-oncogene in the other chromosome piece, thereby making that gene an oncogene. An example, as already noted, was the story of the *BCR-ABL* gene recombination that caused chronic myelogenous leukemia (Chapter 14). (5) A proto-oncogene can become an oncogene when it becomes overactive due to damage or deletion of a tumor suppressor gene that normally limits the activity of the oncogene. These are the most frequent among the many ways that a normal gene, a proto-oncogene, can become an oncogene. In short, anything that causes a gene to send too many "*divide, please!"* messages to the nucleus causes that gene to become an oncogene.

How the first oncogenes were discovered.

The modern story of cancer biology began with the discovery that there were such things as oncogenes. Three separate stories came together in the discovery of the first oncogenes: the *RAS* genes. Three different paths from surprisingly different sources converged in this seminal discovery: (1) mouse leukemia viruses; (2) mutant fruit fly eyes; and (3) gene transfer in human cells. Each of those stories is remarkable in its own way. Each of them came from a different experimental and conceptual background, and their profound relevance to human cancer led to surprising and dramatic changes in cancer cause and treatment concepts. One of the most astonishing discoveries came from the studies of mutations in eyes of fruit flies: who would have guessed that those studies (which might have qualified for Senator Proxmire's "Golden Fleece Award") would lead to discovery of a major human oncogene? (Senator William Proxmire gave 168 or those dubious awards from 1975 to 1988 for projects that he considered to be a gross waste of taxpayers' money.). The remarkable paths from mutant fruit fly eyes and from mouse leukemia viruses, however, will be told in the chapter about the RAS oncogenes (Chapter 18). This chapter begins the story of how the oncogene concept came to be discovered.

Discovery of how to transfer genes from one human cell to another.

It used to seem incredible that it would some-day become possible to transfer genes from one human cell to another. Gene transfer, both natural and experimenter-induced, had been well-known in bacteria and bacterial viruses (bacteriophages) for many years, but attempts to produce it in human or other mammalian cells all failed, until ---

In 1962, a paper appeared that reported that feat (Szybalska and Szybalski, 1962). It seemed astounding the first time I heard of it at a conference in 1961. Waclaw Szybalski and his wife, Elizabeth Hunter Szybalska (Figure 15.1), reported that they had transferred gene DNA extracted from one culture of human cells into the genome of a different culture of human cells. They succeeded in transmitting a gene that coded for an enzyme in the donor cells to recipient cells that were deficient in that function. Although this phenomenon of gene transfer was well known in bacteria, it was a *tour-de-force* to demonstrate it in mammalian cells (Szybalska and Szybalski, 1962).

A few years after that report, the literature had still remained silent: there were no further reports to confirm that astonishing result. I asked Waclaw about it at a conference. He replied (in effect), "Well, it was a difficult experiment and hard to get reproducible data."

Years later, it turned out that the difficulty was a surprising detail that caused the method they used to fail much of the time. When researchers prepared DNA from donor cells for gene transfer, the solutions often became cloudy, because some of the DNA precipitated. The investigators did not like those cloudy solutions and either clarified them or discarded them. It came as a big surprise when researchers eventually noted that the more cloudy the DNA solution, the better the gene transfer worked. When the method was optimized to make the most effective DNA precipitate for uptake into cells, the method became routinely successful. It was the tiny DNA particles of the precipitate that were taken up by the cells, allowing the DNA to enter the recipient cell's genome. It was that long-time prejudice that chemists had against cloudy solution that actually impeded an important discovery.

I am reminded now of an NIH lecture by Nobel Prize winner Albert Szent-Gyorgyi (I think it was in 1958), who mentioned his discovery of the chemical basis of muscle contraction. The discovery, he said, had been hampered for many years by scientists' prejudice against cloudy solution: when researchers added calcium (which was known to initiate muscular contraction) to an extract from muscle, the solution became murky and the scientists would throw it away in disgust. "But," said Szent-Gyorgyi (in effect), "when I saw that precipitation in 1938, I imagined a muscle contracting," which led him to discover the chemical key to muscular contraction: the actin and myosin proteins, and their calcium-induced binding, which is what causes muscles to contract, but in solutions of actin plus myosin produced those precipitates.

It took 16 years after the Szybalska and Szybalski report before DNA transfer between human cells became routinely successful. Michael Wigler, who was then still a graduate student at Columbia University, used a calcium phosphate-DNA co-precipitation technique that worked well in transferring DNA into recipient cells and into their chromosomes (Wigler et al., 1978). Wigler and his coauthors however seem to have been unaware of the earlier work by Szybalska and Szybalski, which my account here may help preserve in the historical record. After Wigler's report in 1978, many laboratories started using that method to transfer genes from one cell type to another (Malumbres and Barbacid, 2003), which opened bright new vistas for research, including the direct identification of oncogenes.

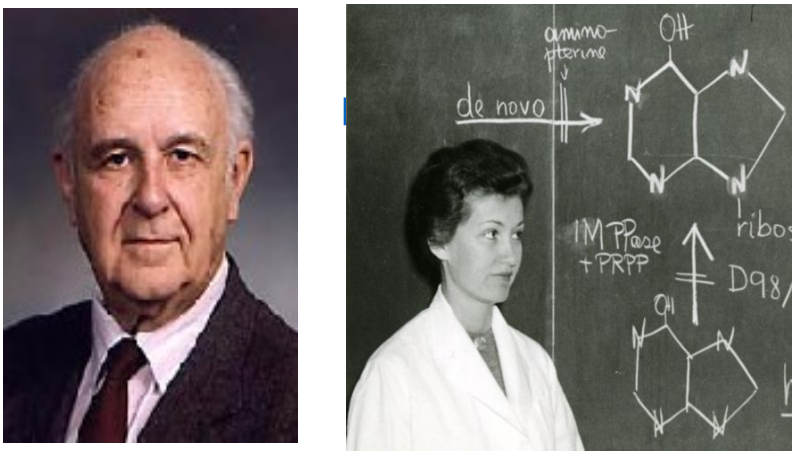

Figure 15.1. Waclaw Szybalski (1921-2020) and Elizabeth Hunter Szybalska (1927-2015), the husband-and-wife team who for the first time accomplished gene transfer between human cells (Szybalska and Szybalski, 1962).

Discovery of oncogenes by gene transfers.

In 1978, about the same time that Wigler's method of gene DNA transfer was developed. Robert A. Weinberg (Figure 15.2), at the time a new faculty member in MIT's Biology Department, had a bold vision (one of many bold visions during his career). He thought that DNA extracted from cancer cells could be transferred into non-cancer cells and cause them to become cancer-like (Shih et al., 1979). He thought that this seemingly far-out idea might succeed using the calcium phosphate DNA co-precipitation technique to transfer DNA and its specific function from one cell to another. The experiment was designed to test whether a gene from a malignant cell could cause cancer in a non-cancer cell. If the test succeeded, it could lead to a major advance in understanding cancer.

However, the experiment was difficult to carry out and was thought to have a low chance of success. No one in Weinberg's lab was willing to undertake it because success seemed unlikely, and his junior doctoral level staff members needed a research success to propel them to their next jobs. I guess his junior researchers thought their mentor's idea was far-fetched. Then, it so happened that a pre-doctoral student Chiaho Shih appeared, looking for a new research project. Shih undertook to carry out Weinberg's idea, and within a few months succeeded in this world-famous accomplishment (Shih et al., 1979; Weinberg, 2011).

The gene transfer from cancer cells to non-cancer cells caused the latter to grow excessively on the surface of a glass dish, producing areas of piled up cells (Figure 15.3). Weinberg was astonished that the experiment actually worked and that a tiny amount DNA from cancer cells could induce cancer-like behavior in non-cancerous cells. It seems

that Weinberg himself may have thought his own idea to be far-fetched and was actually surprised by the astonishing result. After that ground-breaking success, there was no difficulty finding young researchers enthusiastic about carrying these studies forward, which led to their finding the notorious *KRAS* oncogene in a human colon carcinoma (McCoy et al., 1983). The *KRAS* oncogene will be a protagonist in Chapter 18.

Weinberg and his colleagues also showed that DNA from cells that were made cancerous by treatment with a chemical carcinogen could be transferred to non-cancer cells and make them cancer-like. It seemed, therefore, that carcinogens caused changes in some gene or genes that made cells cancerous. In modern language: the discovery was that carcinogens could mutate certain normal genes and cause them to make the cell cancer-like: carcinogens seemed capable of converting proto-oncogenes to oncogenes.

Weinberg tells how the oncogene work got started (Weinberg, 2011). He had returned to MIT, where he had obtained his undergraduate and doctoral degrees -- in part because he would learn much there by working with Nobel Prize winner David Baltimore. Weinberg tells the story of how the critical experiments were carried out by graduate student, Chia-Ho Shih, who came to his lab and agreed to undertake this challenging work that Weinberg's other junior scientists were reluctant to do, because they felt it was not likely to be successful. When these high-stakes experiments actually seemed to work, they aimed for definitive proof by carrying out the experiments in double-blind fashion that, in Weinberg's words, "yielded unequivocal evidence of transforming sequences in the DNA of chemically transformed cells and later in the DNA of a variety of human tumor cells — results that turned out to be most consequential in my own research career."

(When I use the word "proof" in these writings, I am aware that science cannot prove anything beyond doubt. "Proof" comes from Latin *probare* or *proba* to test. Thus "proof" can be taken to mean crucial confirmatory evidence.)

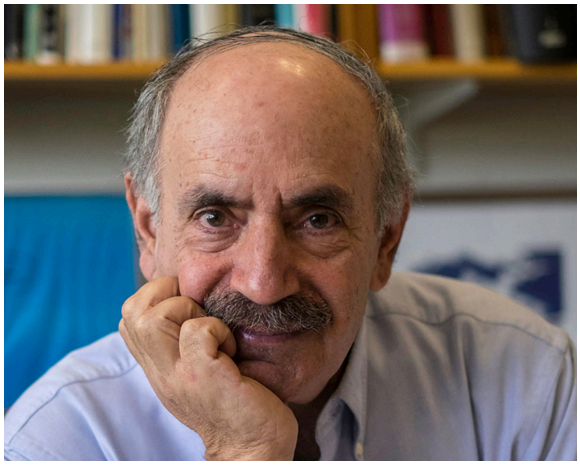

Figure 15.2. Robert A. Weinberg (1942-), discoverer of oncogenes. (Picture from MIT website.)

Message of that early success flew rapidly across the Charles River from MIT to the Sidney Farber Cancer Institute of Harvard Medical School, where the new oncogene methodology was enthusiastically taken up in the laboratory of Geoffery M. Cooper (Cooper, 1982; Cooper et al., 1980; Krontiris and Cooper, 1981).

The methodology was applied to carcinogen-induced mouse cancers, human cancers, and cancer cell lines. The researchers purified DNA from cancers or cancer cell lines and applied it as a DNA-calcium phosphate co-precipitate to recipient non-cancer cells growing on the surface of a plate. Normal cells limited their growth to a single layer of cells. The DNA from cancer cells, as already mentioned, caused some of the recipient cells to multiply excessively (Figure 15.3). As evidence that the foci of piled-up cells were cancer-like, the researchers showed that foci were produced by DNA from cells that had been exposed to carcinogens and not from unexposed cells. Moreover, DNA from cells of a focus was highly effective in producing foci in cultures of non-cancer cells. The cancerous nature of the foci cells was later confirmed by showing that cells from those foci produced tumors when injected into mice. That led cancer researchers to think that at least some and perhaps most cancers arose from one or more mutations in the cell's genome.

Geoffrey Cooper and his colleagues soon found "transforming" genes in several animal and human cancers (Cooper, 1982). ("Transforming gene" meant that DNA containing the gene produced foci of over-growing cells, and that cells from such foci caused cancer in animals.) They found that some of the transforming genes in the recipient cells had DNA sequences resembling the *RAS* genes of Harvey and Kirsten sarcoma viruses, as will be related in the Chapter 18 (Cooper, 1984; Cooper and Lane, 1984) (Der et al., 1982).

Further studies in several laboratories identified three *RAS* genes (*HRAS*, *KRAS*, and *NRAS*) in normal and cancer cells of humans and rodents. They found altered or mutated forms of those genes in many different types of cancer and suspected that the genes contributed to the cause of the cancers. *RAS* genes were found in all vertebrate cells examined, and also in yeast, highlighting the importance of these genes in the control of cell proliferation of very different creatures. Studies in many laboratories over many years disclosed a large number of different oncogenes responsible for many kinds of human cancers. Those cancer-causing oncogenes evidently were mutated versions of the normal *RAS* genes.

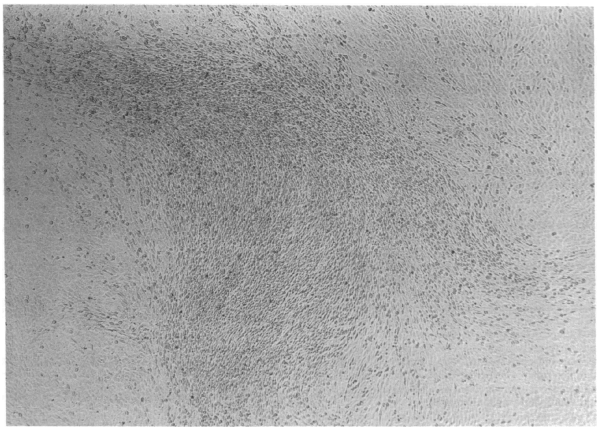

Figure 15.3. Example of an area of cell pile-up caused by excessive multiplication of cells that had received DNA from cancer cells. Normal cells on the surface of a dish grew to form a single layer of cells. When DNA from cancer cells was added to the plate, some of the cells took up the cancer DNA, which induced them to overgrow and pile up in multi-cell layers. One such pile up focus is shown here as an area of high cell density. Cells from such foci produced cancers in animals, confirming that the DNA had caused the cells to become cancerous (Krontiris and Cooper, 1981).

References

Cooper, G.M. (1982). Cellular transforming genes. Science *217*, 801-806.

Cooper, G.M. (1984). The 1984 Walter Hubert lecture. Activation of transforming genes in neoplasms. British journal of cancer *50*, 137-142.

Cooper, G.M., and Lane, M.A. (1984). Cellular transforming genes and oncogenesis. Biochimica et biophysica acta *738*, 9-20.

Cooper, G.M., Okenquist, S., and Silverman, L. (1980). Transforming activity of DNA of chemically transformed and normal cells. Nature *284*, 418-421.

Der, C.J., Krontiris, T.G., and Cooper, G.M. (1982). Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proceedings of the National Academy of Sciences of the United States of America *79*, 3637-3640.

Krontiris, T.G., and Cooper, G.M. (1981). Transforming activity of human tumor DNAs. Proceedings of the National Academy of Sciences of the United States of America *78*, 1181-1184.

Malumbres, M., and Barbacid, M. (2003). RAS oncogenes: the first 30 years. Nature reviews Cancer *3*, 459-465.

McCoy, M.S., Toole, J.J., Cunningham, J.M., Chang, E.H., Lowy, D.R., and Weinberg, R.A. (1983). Characterization of a human colon/lung carcinoma oncogene. Nature *302*, 79-81.

Shih, C., Shilo, B.Z., Goldfarb, M.P., Dannenberg, A., and Weinberg, R.A. (1979). Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin.

Proceedings of the National Academy of Sciences of the United States of America *76*, 5714-5718.

Szybalska, E.H., and Szybalski, W. (1962). Genetics of human cess line. IV. DNA-mediated heritable transformation of a biochemical trait. Proceedings of the National Academy of Sciences of the United States of America *48*, 2026-2034.

Weinberg, R.A. (2011). Hunting the elusive oncogene: a stroke of good luck. Nat Cell Biol *13*, 876.

Wigler, M., Pellicer, A., Silverstein, S., and Axel, R. (1978). Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. Cell *14*, 725-731.