



HAL
open science

Owners profile: the molecular origin of human cancer in history

Enrique Wulff Barreiro

► **To cite this version:**

Enrique Wulff Barreiro. Owners profile: the molecular origin of human cancer in history. 6th European Society for the History of Science Conference, Lisbon 2014, Sep 2014, Lisbon, Portugal. hal-01219354

HAL Id: hal-01219354

<https://hal.science/hal-01219354>

Submitted on 22 Oct 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Owners profile: the molecular origin of human cancer in history*

Enrique Wulff Barreiro¹

Abstract: From 1977 to 1980, the search for genes capable of inducing genotype and phenotype alteration was successfully introduced by DNA-mediated gene transfer experiences. The next step was the purification of the first mammalian gene by genetic means, in 1980. And by 1981, the first cloning of a human oncogene took place. Indeed, when the epochal discovery was made, in 1982, that the functional activation of a human oncogene was caused by a single point mutation, the significance of the presence of Spanish scientists in this pioneering work acquired historical perspective. Evidence that the interaction of a carcinogen with a ras proto-oncogene creates the event that initiates the tumor formation was found to be a major achievement. And a group of Spanish researchers, around the biochemist Severo Ochoa, played a main role in this decoding of the initial human carcinogenesis processes. But in spite they solved the mystery of unlocking the truth on the molecular nature of human oncogenes, their eponymous vindication in relationship with the first steps of oncogenetics was not strong enough. To explore this symbolic reality, a sentiment analysis was made of the point or points where the top stories and events were co-referenced in the documents. Also these data provide an illustration of the emergence of oncogenetics taken on the scale supplied by co-citation analysis to understand the history of science.

Keywords: Oncogenetics; Cancer; History of biochemistry; Co-citation analysis; Point mutation; Sentiment analysis

* A version of this paper was a contribution to: 6th International Conference of the European Society for the History of Science (Lisbon, Sep. 2014).

¹ Marine Sciences Institute of Andalusia, Spanish National Research Council (CSIC), Puerto Real (Cadiz), Spain.
Email: enrique.wulff@icman.csic.es

Introduction

The elucidation of the nature of the oncogene, its real function that would be conserved through evolution, needed screening protocols to ferret out these cancer genes from tumors. If the induction of cancer through the transformation of cells by viruses dates back from the early sixties, like the discovery of the genes (ras genes) transforming rat sarcoma viruses, it was not until 1977 that the methods of gene transfer were successful by using total vertebrate and mammalian DNA [PELLICER, 1986]. Àngel Pellicer had achieved this historical transfer of the thymidine kinase (TK) gene from herpes virus into a cell line of mouse, and Mariano Barbacid figured out that a mutation of ras was responsible for the tumor. Thanks to the development of gene transfer technologies, particularly since the advent of recombinant DNA technology, could be fitted to describe the ups and downs in the pulse of oncogene and mutation, in particular when Barbacid and Pellicer were preceded in the 1989 Nobel Prize for Medicine or Physiology by other scientists from the US who also worked on oncogenes.

In this contribution a widely used technique, the genetic transformation of eukaryotic, is historically approached as a most central matter in the onset of oncogenetics. A strategy that is used to generate a reversal one which ultimately sought to make sense of the genes and proteins responsible for cancerous transformation and yielding more effective therapies. Relying on which Pellicer's work with transfection was communicated to Manuel Perucho and Barbacid in summer 1978, and they realized that animal genes could be transferred from cell to cell and expressed with ease. This Spanish people in the early eighties oncogenes wars - from the developer of the main technique (Pellicer) to the couple of workers (Perucho and Barbacid) who for the first time identified the genetic responsibility for the transformation and cloned the human transforming gene - were some of the kindest and most caring people in the biochemical community in the US. To disparage their efforts those who have followed the 1989 Nobel track involved in heated discussions their significant accomplishments. For the task of the historian of science and technology willing to measure this doubtless influential tradition in incipient oncogenetics, taking the grand sweep in statistical historiography is good both in this particular science and for this particular country.

The Spanish team with which Barbacid raised the question that caused him to establish the molecular base of human neoplasia comprised E. Santos, D. Martín Zanca, and V. Notario. The group of Spanish researchers that worked with A. Pellicer, involved in this decoding of the human carcinogenesis processes, was composed by I. Guerrero, V. Corces, P. Calzada and A. Villasante. M. Perucho engaged in a group which included as Spanish collaborators C. Almoguera, C. Lama and J. Jordano, focused on the characterization of human and murine cellular oncogenes and provided a method to detect mutations in the genes transcripts.

In 1989, the Nobel Prize in Physiology or Medicine was awarded to the discovery that oncogenes in animal tumor viruses are derived from cell genes (proto-oncogenes) [NEWMARK, 1989]. Ultimately, by the fact that normal cells contain genes that may cause cancer if they are altered [GARFIELD, 1990]. The Nobel Committee credited the article [STEHELIN, 1985] published in Nature, where the prize winners reported on the presence of DNA closely linked to the src oncogene of the avian sarcoma virus as part of the genetic material of the chicken cells [NEWMARK, 1989]. In one of his rare references to this work, M. Barbacid suggested that this intelligence of the cellular origin of some viral oncogenes, warned scientists about the possibility that there were also oncogenes associated to neoplasms induced by non viral agents [BARBACID, 1990].

M. Barbacid and A. Pellicer, in 1983 and 1984, found the role played by the ras oncogene activation in human neoplasia and documented it in various carcinogen-induced animal tumour model systems. The first tested chemically induced ras oncogenes activation, through N-nitroso-N-methylurea, of mammary carcinoma in rats [SUKUMAR et al., 1983]. The second obtained a similar result by gamma radiation in brain and thymic lymphoma, also in rats [GUERRERO et al., 1984]. The two used ras oncogene activated by carcinogens with the same mutations than those present in human tumours. And Barbacid received in 1988, the considered by the scientific community as the highest prize in cancer research, the Josef Steiner Foundation (University of Berne) Award for providing evidence that the interaction of a carcinogen with a ras-proto-oncogene creates the event that initiates tumor formation. While Barbacid found a MNU-specific glycine to aspartic acid mutation at the H-ras gene in rat mammary tumors, Pellicer identified a cytosine to adenine transversion as the activating mutation in N-ras associated to hematologic malignancies. Both authors discovered mutations as responsible for the malignant activation at the exonic sequence. The modest fact that a single mutation could induce the development of cancer, the fact that ras oncogenes were not a 'laboratory artefact', took the scientific community by surprise, and led oncogenetists to a heated discussion on its sufficiency or necessity for cancer.

The personal perspective of a Nobel Prize laureate ensures the closest attention about a small segment of a very big chase. Severo Ochoa's description, specially in the United States, as the biochemist of the biochemists and the ideal of what the true scientist should be [SRINIVASAN et al., 1979], highlights his heritage and identity as a key decoder of the genetic code, because this project turned out to depend critically on biochemical tools such as his enzyme (polynucleotide

phosphorylase, with which he produced ribonucleic acids and obtained 1959 Nobel Prize in Physiology or Medicine). Working in the New York University School of Medicine until 1974, he trained specifically to Spain bounded biochemists, in a sufficient number indicative of a scientific school.

S.Ochoa presented some of the contributions by M. Barbacid, and M. Perucho to the National Academy of Sciences. And at the same time he knew their doctoral dissertations advisors D. Vázquez, M.L. Salas and J. Salas. The act of working with a Nobel Prize has brought about, along the history of the award, a greater amount of prizes and a largest number of young people between those who received it [ZUCKERMANN, 1967]. The US government owns the royalties and license uses of these three Spanish scientists. But the 1989 Nobel Prize winners were a second literature that found its way to the award by making use, in terms of scientific policy, of practices own to the management of transfer of scientific results.

So the pioneering nature of these Spanish researchers work, in a highly competitive research race, was exposed to the science stratification that existed in the US at the beginning of the eighties, to the fragmentation awaiting any innovative idea, when the conditions for its eponymic vindication are not enough strong. And the guarantee that was involved with S. Ochoa presenting the works was not effective. After the 1989 Nobel award, the authority of the scientist M. Barbacid fell upon the laureates (since the attribution of the Prize both have been successively directors of the National Institutes of Health). In the US, the mention to Mariano Barbacid acquired the unfair nature of a memorial practice; referred by the official speeches and festivities [MARX, 1994]. The awarded research program imported the discoveries of the program that never climbed the podium, and sets the diffusion patterns for the scientific activity of M. Barbacid. The 89 Nobel winners anticipated the Spanish scientists.

Methodology

A question on which is the universe of resources that we have taken to portray the results of earlier studies in oncogenetics, is convened by the gauged ensemble of publications. Right in this sense, there is any publications taken to get answers to the scientific community on the emergence of oncogenetics from virus work, the study of eukaryotic transfection, and the somatic mutation theory of cancer (see appendix). It can be thought along these lines that, those scientists who participated in the oncogenes races of the earlier eighties underlined their visibility and independence, so striving to unlock their contributions to the best point of acknowledgement. There are definitely many things that can be done with these regular instances research colleagues express their influences towards each other. So that the methods employed to screen for data to draw models to illustrate the ideas about oncogenes could be useful in solving the problems necessary to justify this shifting, and developing area in its early times.

Typically there are two types of assets that can be recognized as sensible when following the development of a scientific specialty. One type is focused on catching the intellectual problems and processes on which a kind of cross-over indicators can be built. And the other one is based in attention as recorded by citations, basically figuring out whether a scientist has more citations than normally expected for a given situation. And the reason why it is interesting is that certain scientists results have been in use more than others. There is here a large correlation to size, so your different teams get more citations than some more outside investigators out there. And the way the possibilities of almost all these tools can be applied, very much comply with your imagination really.

We look at enhancing, can you say, reversal strategies based on time distribution of ideas as usual, we have looked at the concept of oncogene amplification during tumorigenesis, we have looked at how fundamental effects are between top events. There are a lot of different things that can be done here. By using co-reference analysis that considers the family matrix, timing and relevance of the couple of concepts considered as a pair, top-events defining the relationship between the co-referenced pair of ideas and, portraying the link to the underlying top story to summarize the relationship.

In terms of earnings per author in scientific projects in their community, it would be definitely interesting to see how a scientist is portrayed into the scientific papers for a series of years and to see how that modify how the scientific community reacts when the actual learning is announced. Co-citation activity plot, looking at analyst reading moving shifts in intellectual focus, is particularly adequate in this sense. You may be able to use news in some way to position yourself before intellectual changes occurred. Showing how a scientific interest grows, a model runs into trouble or important predictions are disconfirmed by experiment. So in the order of coming to identify when there is a likely scientific event rightly coming out, you may look at what kind of patterns of contents tends to trigger any action from investigators and, then you may be able to position yourself before hand. The positive substantial skew with which co-citations really correlates with historical processes, is definitely true when you look at the question of time. Here the relationship is about to reach the most bottom of the 1973 new transfection protocol by Graham and van der Eb's calculations and it scrutinize the scientific proportions of 1981 and 1982 keystones. By looking into the macro-space we have piloted a methodology to

relate a global index, like the co-reference indicator, with a more traditional input-output connectivity, like co-citation analysis.

Intellectual problems and processes

We take a technical approach to very fundamental research problems. However, research on ras genes is justified because they play a fundamental role in basic cellular functions due to their phylogenetic origin, in the early eighties the focus was on their role in neoplastic development. Thus we look for cross-overs between the first time a human-tumor-derived cell transformed mouse cells and, the surprise that single point mutations were behind oncogenic ability to induce tumors. The purpose here is not simply to show the chronology within which these discoveries occurred but rather to indicate the pattern in which they occurred, since that pattern can indicate (roughly) the several stages to which the intellectual problem has developed. These phases correspond roughly to the processes where the oncogenes had been detected in human and other animal tumors through transfection, and the point mutation work at the twelfth codon presumably affecting the ability of the protein to contribute to human tumors.

In each of the stages (see Figures 1 & 2), a cross-over from below and above translates into a bullish view, and a cross-over from above and below translates into a bearish view. A cross-over is considered as driven by co-references, in particular, the co-reference indicator was created as a simple rate of journals to time, applying a span of time sufficient to satisfy the given purpose of the paper examined. A simple strategy is considered in which we are in the knowledge space of the working literature all the time. High density of useful material is entered like signals to generate a significant history. It must be emphasized that for some of these given intellectual problems, positions were overlapped and we have hold them. In terms of this analytical scheme a fixed time horizon is applied and all grouped papers are created equally.

The tide of excitement swept up in the currents of history, in the competitive races for the oncogene of the early eighties. The characterization of human and murine cellular oncogenes was a race, and the search for the function of ras was definitely a race.

Intellectual Problem: Study of the isolation of a human oncogene constituted Manuel Perucho (a Cold Spring Harbor scientist) central intellectual problem until the autumn of 1982. The response of biochemists to the fact that cellular genes (proto-oncogenes) may become activated by somatic mutations that mimic the changes imposed upon these loci during endogenous retroviral transduction, was to show in the transfection system that methylated and unmethylated DNAs were equally well accepted by the recipient cell. By that it meant the fortunate rediscovery of ras genes and, in the summer of 1981, gene transfer assays revealed for the first time that a defined genetic element was responsible for the transformation of NIH-3T3 cells. Based on this, after looking at Figure 1, it turns out that the work performed was science as good as what was going on at Bethesda, where Barbacid had created a new Laboratory of Cellular and Molecular Biology at National Cancer Institute (NCI). Both teams were in a race now; they wanted to clone the bladder oncogene.

Intellectual Problem: Since Mariano Barbacid had branched out from virology, he was backed by the right immunological approach to generate transforming viruses, from several mammalian species, which transformed activities were stably associated with oncogenes picked up by these retroviruses from their genomes. This homology between ras genes of rat sarcoma viruses and activated oncogenes contained in human tumors showed for the first time the bridge between the pioneering work carried out with tumor viruses during the 1960s and 1970s and the realms of those working on human cancer [MORANGE, 1997]. We will return to the nature of this bridge later on, across the standard rodent models provided by Àngel Pellicer (Figure 2). With the evidence that the ras oncogene was some sort of a common denominator for cancer, scientists had to determine how in the evolution of cancer the ras gene degenerated from a healthy, vital cellular gene into a miserable oncogene where the mutation is dominant. Taking the scientific community by surprise a single ras gene proved able to induce significant transformations in NIH-3T3 able to confer on them the ability to induce tumors in nude mutations. Mammalian ras genes acquired their properties to induce malignancy by single point mutations in codons 12 [SULLIVAN et al., 1983].

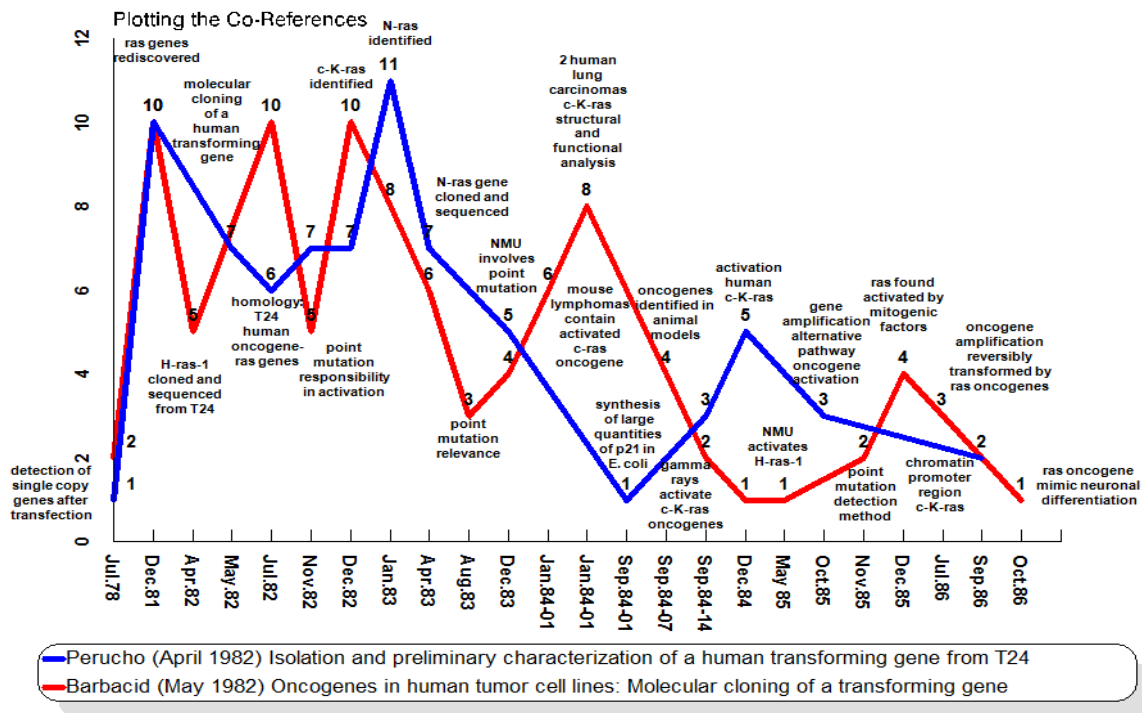


Figure 1. Plotting the Co-References between the between the spring of 1982 results on the existence of transforming genes in human tumor cells.

To get a better understanding of the nature of the relationships between these two intellectual problems, we consider them both when the target was the first three ras genes identified in the mammalian genome as well as when the related single point mutations were targeted. Both problems are required to have a high related mention in the history of oncogenetics. To see if we are actually able to identify any impact on the course of events, we turn to the plotting of the co-references against their return relationships between July 1978 and October 1986 (Figure 1). With 21 top stories and 167 co-references, and a Hit Ratio of 11, on average the strategy generates 6.2 sized-groups. In processing this information Perucho is hypothesized the key driver of the race (he stands out for 45.5% of the return on event; Barbacid (40.7%), Pellicer (13.7%)).

To understand the relationships in greater detail we can either consult the top events or either we consider the cross-overs between the two programs in the co-references staging. Comparing the co-references spikes, looking at the first top story back in December 1981, we find that this first spike claim the identification of the first member of the ras gene family. Otherwise we do observe that the first common assay for the detection of mutant ras alleles (based on the ability of these alleles to transform the mouse NIH/3T3 cell line, which meant the rediscovery of ras genes), uttered in scientific circles in December 1981, had a common impact in the co-reference scenario from the point of view of both programs. On the existence of transforming genes in human tumors, its clonage as an oncogene (H-ras-1) was carried out by the same team and published in April 1982. A couple of months later, in light of this same T24 bladder oncogene (homologous with Harvey sarcoma virus ras gene (H-ras)) discovery, the result has come out of that row from the retrovirologists side. This positive performance drives a particularly visible cross-over between equally faster indicators from both approaches. The bearish trend recorded in the characterization of oncogenes research front cross the bullish trend faced by the oncogenes and the genetics of human cancer research front. We take a look again, to highlight the fundamental homology between the T24 human bladder oncogene and the ras transforming genes coming from the BALB- and Harvey-murine sarcoma viruses. The event is made by correlating the biochemical program with the oncogenetics.

Within mutation research getting access to using data from the high-expressing human bladder carcinoma cells to support the point mutation case, at the which cloning things were quite down for the second time, was a result that can be clearly appreciated by observing the second cross-over. Then things begin to hit up again with the identification in human lung and pancreas human carcinomas of a common oncogene which shares sequences with the onc gene of the Kirsten strain of murine sarcoma virus. Also it was obtained that about 15% of the human tumors contain activated oncogenes (December 1982). And with the human neuroblastoma cell line transforming gene identified and found to be designated N-ras (January 1983). To support the case a few months later N-ras gene was cloned and sequenced (April 1983), which was the second decision in favor of characterization's group, followed by a falling-out, an indicative intent for appealing results from the

oncogenetical program searched to consolidate Severo Ochoa decisive recognition (August 1983). A fifth cross-over has been added in the framework of the identification of oncogenes in a NMU induced point mutation (Dec. 1983).

An actual attraction followed along a month later, until both programs claimed for the January 1984 result, by the NYU pathologist Àngel Pellicer, relative to the identity of activated oncogenes in a carcinogen-induced animal tumor model. Then things went up again this time holding a position, along this same month of January 1984, by a second hit currently reassuring the scientific community after the identification of a mutated *kras* oncogene in a tumor biopsy from a lung cancer patient. Two quick mentioned events refer the synthesis of large quantities of p21 in *E. coli* (Sep. 1984, 01) and N-gene identified in animal models (Sep. 1984, 07). There is a "special noise" in a particular event for September 14, 1984, novelty provides information about another cross-over. The success of the NYU pathologist rodent models appears again, as the evidence that gamma rays could be the initiating carcinogen able to activate *c-K-ras* oncogenes through a somatic mutation and to induce lymphomas in mice. Two months later the activation of human *c-K-ras* makes again correlate both research programs (Dec. 1984).

May 1985 shows NMU activates *H-ras-1* and October that gene amplification is an alternative pathway for oncogene activation. And finally by November 1985, Ochoa was able to settle the case by his support to a clinically oriented point mutation detection method, then expression of the positive association between *ras* genes and mitogenic factors. This event tailors specifically the next cross-over. Little before that *ras* was found activated by mitogenic factors with loss of the N-*ras* allele in a mouse thymic lymphoma (Dec. 85). Then amplification, a consistent sign of a more dangerous type of malignant transformation, was proved to be a reversible process that depends on the relative abundance of oncogene sequences, as can be observed across July 1986. Chromatin promoter region, in *c-K-ras*, was described by the characterization program (Sept. 86). At last, it is time to appreciate the building of a new research area, as indicated by the *ras* oncogene mimic neuronal differentiation event (Oct. 1986).

Over the monthly periods considered the number of co-references stories between the two research fronts was ranging between 75 and 87 respectively, consulting the top stories we were able to summarize the relationships in only fifteen cutting lines. From the quantitative point of view incorporating co-referencing allows adequate products picking up, increasing cross-axis correlations, and even the research direction. The top event must provide useful information to understand the relationships and whether to expect positive or negative spill-over effects. The case symbol top events detected over time from the respected research programs included anything from methodological issues, to basic discoveries and to actual, veridic and follow-up acknowledged results; and finally a settlement by Ochoa. Hence, in this case it would have been possible to successfully having model the characterization of cellular oncogenes versus human cancer & viral transforming genes programs as a part of an automated analysis process by simply using the top events together with spikes and using co-referencing.

Let's try to look at another simpler example. In this case we look at news co-referencing between the murine lymphoma system to study the mutated oncogenes and carcinogenic agents (molecular oncology animal models developed by Pellicer) and the activation of distinct oncogenic pathways in the induction of mammary carcinomas by single point mutations achieved by Barbacid. As can be observed that has proved to be a lot of action over time, in fact 25 top stories and 376 co-references between 1978 and 1986, but just three cross-overs. Hit Ratio of 18, on average, the strategy generates 15,32-references-sized groups (Figure 2). We hypothesize that Barbacid did lead the investigations (he gave recognition to 47% of the events; Perucho (32%), Pellicer (21%)).

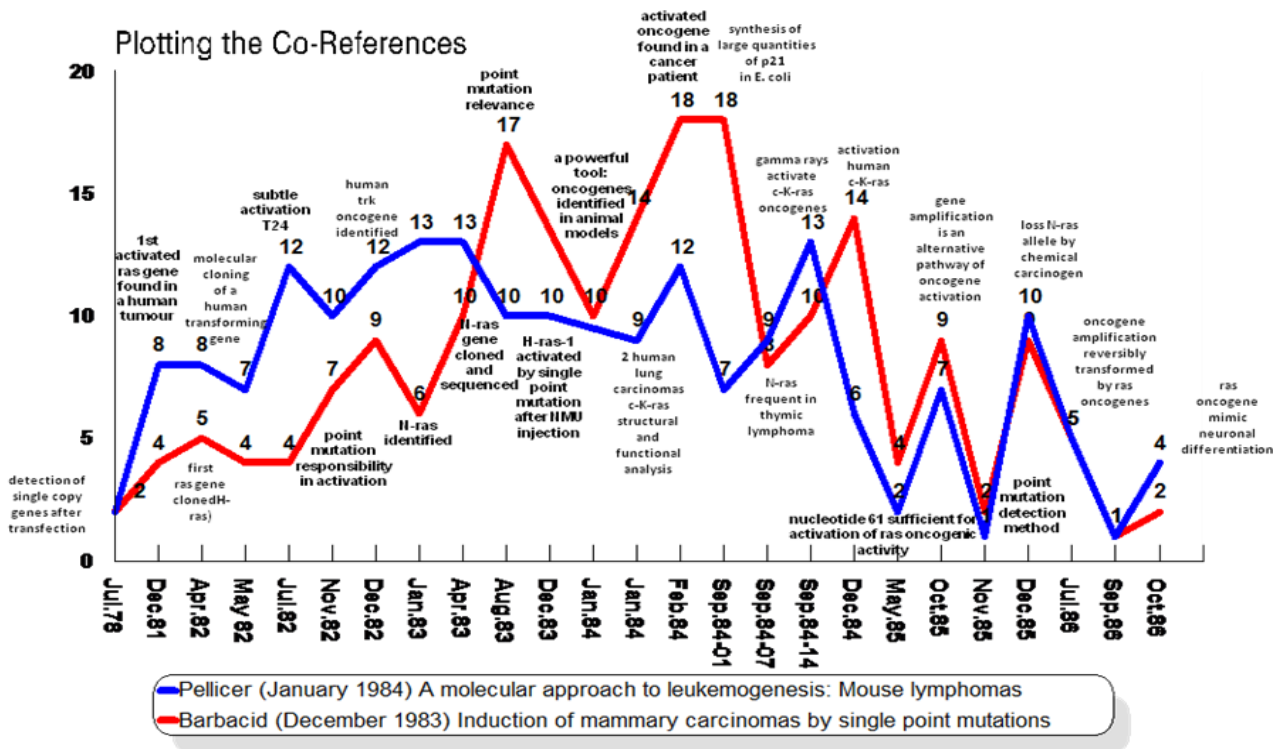


Figure 2. Plotting the Co-References between the ras oncogenes and carcinogen-induced animal tumor

From this Figure 2 information around scientifically linked programs correlations delivers an impressive combination of size and performance, detecting a contagion effect extended to all the events across the temporal chain. All pairs contribute positively to performance. Also, visible at a glance, is that the steep diagonals soars steadily upwards and downwards to the same scientific keystones, specially since September, 1984. And it is probably fair to state that these two researchers would be considered related at least from the summer of 1978. The first time the two programs were co-referenced was when Pellicer shared with Barbacid a landmark experiment on the detection of single copy genes after transfection. Then first release came out that an activated ras gene was found in a human tumor in December 1981. Watching out for the first ras gene cloned (H-ras) allows us to check the T24 human bladder carcinoma oncogene April 1982 result. To understand the relationship between oncogene cloning and identification, we observe the agreement of co-referencing for May-December 1982, including the point mutation responsibility in activation finding by May 1982. There is quite a lot ranging between H-ras molecular cloning from human bladder cells to the thrilling original identification of the human *trk* oncogene. This last contribution was published in December 1982, and for many years it was the sole example of a TK (tyrosine kinase) oncogene activated in a solid human tumor, been the target of the first cross-over, the characterization of the transforming gene of neuroblastoma cells (N-ras). Ochoa communicated the spontaneous acquisition of transforming properties by a human proto-oncogene, mediated by a single point mutation, and occurred at the same nucleotide. This approach was published in August 1983.

On the date when the scientific community was astonished by the fact that a single injection of a carcinogenic agent conferred its extraordinary power to the oncogenic ras gene (December 1983), a powerful model in the study of leukemogenesis takes all its relevancy, pushing down the co-references by January 1984. In this last case, the event showed a clear increase of the transformed cells following K-ras gene activation induced by radiation. So the early stages of carcinogenesis study in *in vivo* systems, was associated to oncogenes identified in animal models. Also in January 1984, a comparative analysis of the mutation responsible for the activation of the human c-K-ras oncogene in two human lung tumors indicated that point mutations in different tumors are not identical. In February 1984, an activated oncogene found in a cancer patient improved on its own the supply of evidence to reassure the scientific community that ras oncogenes were not a 'laboratory artefact'. After that, we do see an event communicated by Ochoa to the National Academy of Sciences of the US. Top-story was driven by the achievement of the synthesis of large quantities of both normal and transforming p21 proteins. And it resulted from the need of significant amounts of purified p21 proteins to unveil the biochemical role that ras gene products play in normal cellular growth and differentiation, as well as in malignant transformation (September 1, 1984). Looking into the next data we arrive to a common recognition of the N-ras genes (from a thymic lymphoma chemically induced) cloning and sequencing event (September 7, 1984). These two last results are identified with the best scores (18). From there the more close the relationship between both programs, the more time analysis is driven by the same information across the competitive landscape. In fact, for September 14, 1984, we get a

signal looking at gamma rays as activators of c-K-ras oncogenes by somatic mutations. And as an expression of the relative common importance of the reciprocal usages of both programs, the following result refers to the activation of a human c-K-ras oncogene.

Let's take a look at the next examples. Primarily for May 1985, concerning direct proof for the generation of tumors in mice involving genes, a series of experiments revealed that 85 percent of lab rats injected with a mutagenic chemical contracted mammary carcinoma. Whenever we travel from spring to winter 1985, we are presented with October 1985 mechanism, corresponding to the evidence of the malignant transformation and ras gene amplification relationship, that increased amount of normal proto-oncogene proteins might alter the basic regulatory controls of cell proliferation. A detecting point mutation method was revealed by November 1985. And for December 1985, it was time to observe the loss of the normal allele in mouse thymic tumors that contained oncogenic versions of ras genes. We do appreciate the relation between the oncogenetics program and research front code 84-4046, an edge supported by quantitative as well as qualitative alterations in mammalian ras genes concerning the allele loss oncogenic dominance. Besides increasing the number of signals moving beyond strict cross-over strategy, the result showed promise, previously unformulated strategies based on the suggestion that the expression of a minimum level of mutant ras gene products may be critical in tumorigenesis. What increased the likelihood of reversibility of the malignant transformation caused by mutant human ras oncogenes (July 1986). Looking at the performing graphics (Figure 2), we get an information issue as mentioned previously.

Finally, after considering the last cross-over around the chromatin structure of the promoter region (once promoter mutation theory offered an alternative to point mutation), we turn out to the other side of the equation, cellular differentiation, because ras genes can have other effects besides the induction of uncontrolled proliferation, activating the pathways of differentiation. In October 1986, the human cancer research front clearly perceived as a substantial experiment the study on the effects of ras genes on the cells in the neuroendocrine tumor of the medulla of the adrenal glands. These cells can differentiate into neuron-like cells if infected with ras-containing retroviruses or microinjected with oncogenic p21 ras proteins. So demonstrating that the introduction of a ras oncogene was able to arrest growth and mimic the steps of neuronal differentiation. This induction of terminal differentiation in neural cells implied ras oncogenes in a signal transduction pathway commonly used by NGF, a strong result with consequences on the research of the receptors for nerve growth-factor and other neurotrophins.

As we have seen using co-reference data makes easy to get notify of new or reciprocal relationships and, could be available input for a case history management process as to this a more timely, measured and correlation tool.

Emergence of oncogenetics

A modification of the ras protein itself, due to a point mutation in the coding sequence of the gene, was placed by the authors of its discovery in the general framework of cancer studies [MORANGE, 1997]. The outstanding accomplishment (of 1982) was the introduction of oncogenetics as a new period within the history of human genetics, where important intellectual changes occurred on a scale much smaller than a year's span [SULLIVAN et al., 1979]. Co-citation studies have been proved to be a powerful tool sensitive to very rapid shifts in intellectual focus, having used it to ran into this area, we now provide a sequence of the events (Figure 3).

The period from 1973 to 1982 is ideal because it codifies the first set of successful assays in the domain of oncogenicity. Two Dutch scientists, Frank Graham and Alex Van der Eb developed a new technique to introduce foreign DNA into mammalian cells as a means of studying gene functions [MALUMBRES & BARBACID, 2003]. The Dutch scientists had transfected DNA into cultured human kidney cells. First they diluted DNA of low complexity (an adenovirus that causes respiratory diseases in humans) in isotonic saline containing phosphate at low concentration, and after adding calcium chloride, the formation of a calcium phosphate precipitate resulted. Once the investigators put these crystals onto cultured human kidney cells, they incubated the cells for a couple of weeks. Clearly, after that, the foreign genes integrated into the chromosomes of the kidney cells [ANGIER, 2014]. Circumventing the dependence on pathogens calcium phosphate had permitted transfection of foreign genes into the host DNA of cultured cells. Put another way, transfection has had the same effect on cultured cells as infection.

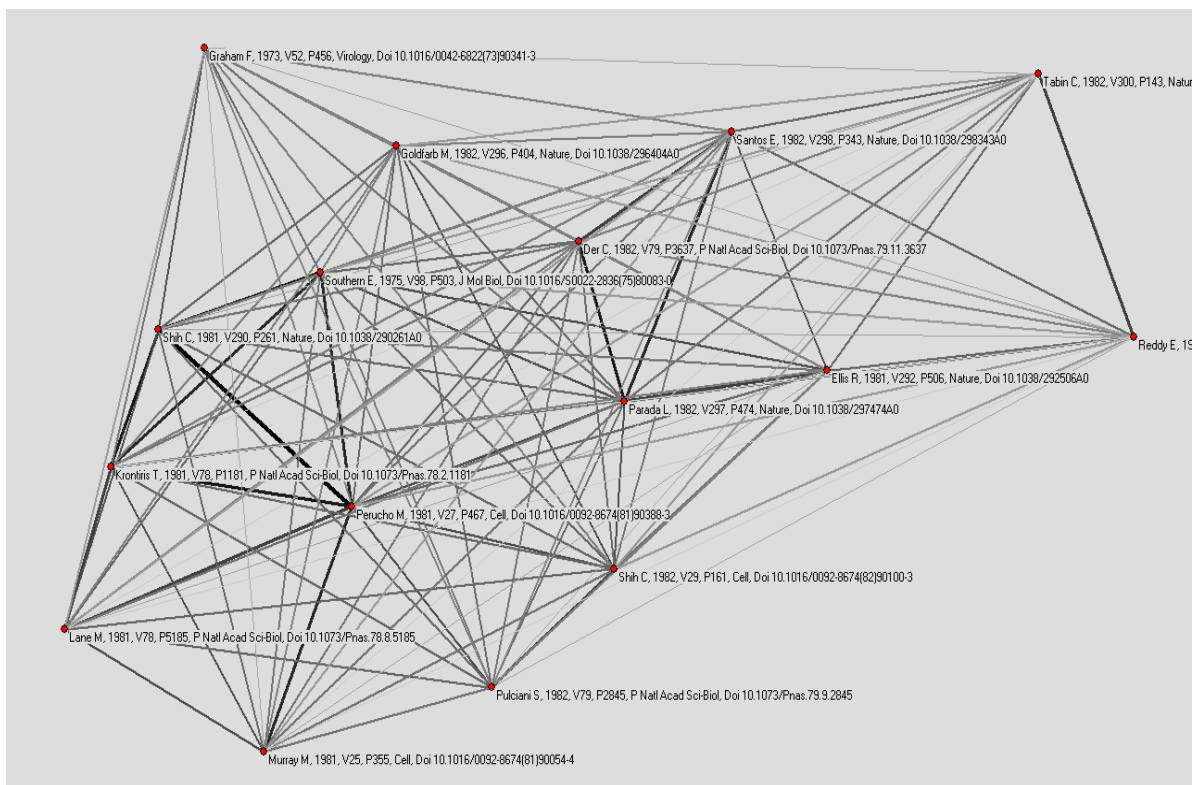


Figure 3. Oncogenetics Co-Citation activity plot for 1973-1982.

However, until 1977, the reference to transfection must be restricted as the methods of gene transfer were successful only when this DNA of low complexity was used [PELLICER, 1986]. Figure 3 shows the status of Graham and Van Eb technique, which we can distinguish in the left upper region, with a commonality of usage (estimated by number of connections) similar to the main discovery in the field, the single point mutation mechanism of oncogenic activation, represented in the right corner (Reddy). The basic concern of this research, beyond understanding the function of ras, was to understand the genetics of human cancer. This more purely genetic concern and one which is more a user of recombinant -DNA technology than a source of that technology, is represented in the right region. All the events and findings of the past in this area, co-exist at a single time in the past, have been placed together at the same moment, in 1982.

Turning first to the left region, mostly a blow-up of the 1981 picture (Figure 3), which we may roughly identify with the characterization of human and murine cellular oncogenes research front, this crucial instrumentality is represented by the hill labelled "Southern E, 1975". A simple method, in that it works with diced DNA transferred to lots of dry paper, Southern blot, the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellular sheets, said also the frontiers within which emerged in time the new research specialty of oncogenetics. This technique was ubiquitous in all the biological laboratories in the context of forming DNA:RNA hybrids and, its need investigated the possibilities of development of genetic engineering. In 1975 Edward Southern, a Scottish biologist, published the recombinant DNA work that he had devised and with which the biochemists involved in the early eighties oncogene races spend many hours preparing various types of blots.

The middle and rather central stage of this region is occupied by Perucho's key paper. The practical concern here is the newly resurrected ras gene model, that determined for the first time the detection of mutant ras alleles (elements called H-, K- and N-ras, implicated in a wide range of human tumours). The most initial assay it was based on the ability of these alleles to transform the mouse NIH/3T3 cell line. In the chronicle of oncogenetics, evidence was offered of a first sign of transformation efficiency using DNAs from different human-tumor-derived cell lines. The cell lines analyzed which gave rise to foci containing the transfected oncogenes were SK-CO-1 in the case of colon carcinoma, and Calu-1 and SK-LU-1 in lung carcinoma; on the charge of DNA derived from a neuroblastoma SK-N-SH line, another member of the ras genes family was identified, later characterized as the N-ras.

In Figure 3 we see that it was Mike Murray, getting a larger link with the central node, who supplied evidence for the same colon carcinoma oncogene (this time using SW480 cell line; also he distinguished a transforming gene associated with a leukemic cell line, HL60). He made a difference, but it was certainly not a stellar accomplishment. On the left, the

triangularity shaped group of concepts concerns also this accurate measure of the transforming potential of the altered ras genes. And, in fact, it treated all the similar results been reported previously, either by Chiaho Shih or by Theodore G. Krontiris and Geoffrey Cooper. Together with Pulciani (in the cluster of Barbacid, but on the right immediate link with this region), these scientists were the inadvertent rediscoverers of ras genes when, using gene transfer assays, they established the existence of dominant oncogenes in humans. Shih's paper in Nature analyzed DNA isolated from mouse fibroblasts transformed by human tumor DNA, and Krontiris and Cooper also had transfected DNA, from human bladder carcinoma cells (although they were published in PNAS, which has a far less circulation (and reputation) than Nature). Shih's initial transfection success had been met with scepticism, hence, in effect, the caveat was solved when he showed transforming activity in NIH-3T3 cells (a mouse line created by G.J. Todaro) infected with DNA from human tumour cell lines. The remaining concept in this region stand for stage-specific transforming genes. Once Cooper leave Weinberg's labo (easy come, easy go), he identified stage-specific transforming genes in MCF-4 mammary carcinoma cells (tx-4). As expressed by the work of Lane, at the left end of the region. It turned out that these genes were specific for the differentiating stage of the tumor cell in which they were identified. These observations were driven from both human and mouse tumors; as such, for instance, tx-4 is present in both human MCF-7 mammary carcinoma cells and in several rodent mammary carcinomas.

From this fairly coherent region a bridge connects to the right side via Channing Der, a postdoctoral fellow in Geoffrey Cooper's laboratory. The ras connection arouse the interest of biologists through his experiments. He used retroviral oncogene probes to hybridize DNA isolated from NIH-3T3 cells that had been transformed with various human tumour DNAs. But, above all, he showed that transfected oncogene were identical to the retrovirally encoded oncogenes. The most straightforward interpretation of his findings was that retroviral oncogenes were derived from cellular genes [VECCHIO, 1993]. The special properties of both Harvey (Ha-MSV) and Kirsten (Ki-MSV) murine sarcoma viruses, according to which they were actually triple recombinants between the replication competent (helper) retrovirus that conferred on them infectious properties, rat cellular sequences of ancestral retroviral origin known as 30S RNA and rat cellular genes (H-ras in Ha-MSV and K-ras in Ki-MSV) [MALUMBRES & BARBACID, 2003], were exploited in the 1981 transduction work associated with Ellis in the right part. So 1982 Der's positive answer to the question of whether or not human oncogenes were related to the cellular homologues of the retroviral oncogenes was based on the description of the molecular clones of viral DNAs previous 1981 description by Ellis.

But the linkage between the regions is one of historical as well as operational dependence. Investigators to establish the functional side of the oncogene, started from the spring of 1982 results, when Goldfarb (he had been a previous member of Weinberg's team), Pulciani and Shih reported on the molecular cloning of a human transforming gene from the T24 and bladder carcinoma cell lines. This substructure, that do group in a rather diffuse triangle at the middle of the Figure 3 center, evolved into the trapezoidal map, at the right of the right region, associated with the ability of a single point mutation to lead to the carcinoma. As a result that led to a heated debate in the oncogene community [DUESBERG, 1985].

But it was not until the autumn of 1982 that the nucleotide sequences of the Harvey- and Kirsten- sarcoma viruses oncogenes (isolated from NIH-3T3 cells) were published. That was in fact the nature of the bridge between regions left and right via Der (Figure 3). A fairly coherent sub-group to the right of center illustrates the relationship between three indepent reports (Der, Parada and Santos) having found similar results. All these three were able to generate an information relative to cells transformed with DNA isolated from EJ bladder and lung carcinoma cells having additional sequences that hybridized to probes specific for the v-h-ras and the v-k-ras oncogenes, respectively. As a consequence of these results, taken together, and before getting into the last section of this graph, the historical data available suggested some questions. Was it possible that nobody thought to hybridize a blot containing DNA from transfected NIH-3T3 cells with a virus ras probe until those days? Another question that comes is if perhaps researchers wanted human cancer to be distinct from virus-induced tumors of rodent?

That nobody thought that those oncogenes transduced by retroviruses could be mutated in human cancer prepared the surprise. A single point mutation was responsible for the malignant activation of ras oncogenes. As it was established that p21 is produced constitutively in all human tissue, revealing an important role of ras genes in normal cell growth. And ras protein is known to need guanine triphosphate, or GTP, to be active, being the role of p21 to transduce molecular signals to the cell nucleus. The simplicity of a single amino acid change in the p21 protein product was too much to take. As a primary event point mutations must occur all the time, every day in every cell, but people do not develop cancer until old age. It was not clear how a single mutation in a membrane-based protein made GTP enzymatic activity so greatly reduced, made ras do in humans enough to provoke the biochemical modifications occurring in cancer cells. Mutations are a major activating mechanism for the ras family genes, mainly in codons 12, 13 and 61, resulting in their conversion from proto-oncogenes to activated oncogenes. As shown vertically, in a roughly linear fashion, two results associated to the mechanism of activation to the right region. This connection is concerned with that human transforming genes were mutant ras genes, and was announced in winter of 1982.

At the right corner on our Figure 3 (Tabin, Reddy), the limited constraint with which ras genes acquire transforming properties, is in contradiction to the prediction that located the alteration in the gene promoter. But the frantic pace of research made a revelation in response to the question on having previously thought that those oncogenes that were transduced by retroviruses could be mutated in human cancer.

Conclusions

The frequent detection of ras-related oncogenes activated by single point mutations is a genetic change detected in about 15% of all human tumors. But there are people thinking they were looked for by seeking a cure for cancer in our time. In the search for basic cancer therapies the biochemical and biological properties of ras proteins shows that oncogenic mutants are the target of immunotherapies searching to induce immune responses against them in patients. There is a way for the history and pathogenesis of cancer to benefit from basic research on oncogenes in terms of prevention, diagnosis and therapy of human cancer.

In order to figure out whether a treatable strategy was available, one having had to precisely timing the connection between human oncogenes, its mechanism of activation, and the ras proteins, watching out for those unexpected events surrounding the identification and subsequent characterization of oncogenes in human tumors, as well as the discovery of the point mutation, helps in mapping factors whose regulatory activity converges. Hence, it is possible to find powerful moves validating the pioneering work carried out with tumor viruses during the 1960s and 1970s. Members of the Ras superfamily of small GTP-binding proteins have been implicated in the regulation of diverse biological functions, including tumor progression. Activating mutations in Ras genes are commonly found in a variety of human tumors. As well as a molecular diagnosis to identify those oncogenes that exert their transforming properties by mechanisms involving gene amplification was put at disposal by Ochoa in 1985. The thirty-three anniversary of the identification of the first human oncogene, makes the area of research into the three closely related proteins, H-, K-, and N-RAS more accountable to virology than ever before.

References

- ANGIER, N. (2014) *Natural obsessions : striving to unlock the deepest secrets of the cancer cell*, Boston, New York, Mariner books.
- BARBACID, M. (1990) ras oncogenes: their role in neoplasia. *European Journal of Clinical Investigation*, 20 : 225-235. doi: 10.1111/j-1365-2362.1990.tb01848.x
- DUESBERG, P.H. (1985) Activated proto-onc genes : sufficient or necessary for cancer?, *Science*, 228 : 669-77. doi: 10.1126/science.3992240
- GARFIELD, E. (1990) The 1989 Nobel Prize in Physiology or Medicine is awarded to J. Michael Bishop and Harold E. Varmus for their contribution to cancer research, *Current Contents*, 22 (16) : 3-10.
- GUERRERO, I., VILLASANTE, A., CORCES, V., PELLICER, A. (1984) Activation of a c-K-ras oncogene by somatic mutation in mouse lymphomas induced by gamma radiation, *Science*, 225 : 1159-1162. doi: 10.1126/science.6474169
- MALUMBRES, M., BARBACID, M. (2003) RAS oncogenes: the first 30 years, *Nature Reviews Cancer*, 3 : 7-13. doi: 10.1038/nrc1097
- MARX, J. (1994) Oncogenes reach a milestone, *Science*, 5193 : 1942-1944. doi: 10.1126/science.7801118
- MORANGE, M. (1997) From the regulatory vision of cancer to the oncogene paradigm, 1975–1985, *Journal of the History of Biology*, 30 : 1-29. doi: 10.1023/A:1004255309721
- NEWMARK, P. (1989) Nobel for oncogenes, *Nature*, 341(475) : 12. doi: 10.1038/341475b0
- PELLICER, A. (1986) Gene purification by transfection methods. In: R. KUCHERLAPATY (Ed.), *Gene transfer*. Plenum Press.
- SRINIVASAN, P.M., FRUTON, J.S., EDSALL, J.T. eds. (1979) *The origins of biochemistry: A Retrospect on Proteins*. New York, New York Academy of Sciences
- STEHELIN, D. (1985) Oncogènes: de modèles simples vers des modèles plus sophistiqués, *Nouvelle Revue Française d'Hématologie*, 27 : 57.
- SUKUMAR, S., NOTARIO, V., MARTÍN-ZANCA, D., BARBACID, M. (1983) Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of H-ras-1 locus by single point mutations, *Nature*, 306 : 658-666. doi: 10.1038/306658a0
- SULLIVAN, D., KOESTER, D., WHITE D.H., KERN R. (1979) Understanding rapid theoretical change in particle physics: a month-by-month co-citation analysis. In: *Information choices and policies*. New York, Knowledge Industries Publications.
- VECCHIO, G. (1993) Oncogenes of DNA and RNA tumor viruses and the origin of cellular oncogenes, *History and Philosophy of the Life Sciences*, 15 : 59-74.
- ZUCKERMANN, H. (1967) Nobel laureates in science : patterns of productivity, collaboration, and authorship, *American Sociological Review*, 64 : 1087-1094. doi: 10.2307/2091086

Appendix

These are the 25 research articles selected for studying this case history. The specifications consist of: number of citations (until September 21, 2014); articles communicated by Severo Ochoa; together with the research fronts papers from which the co-references have been plotted.

Nº 1

Wigler, M.; Pellicer, A.; Silverstein, S. and Axel, R.: "Biochemical transfer of single-copy eukaryotic genes using total cellular DNA". *Cell*, 14 (Jul. 1978), 725-31. doi: 10.1016/0092-8674(78)90254-4
[1161 citations.]

Nº 2

Perucho, M.; Goldfarb, M.; Shimizu, K.; Lama, C.; Fogh, J. and Wigler, M.: "Human-tumor-derived cell lines contain common and different transforming genes". *Cell*, 27 (Dec. 1981), 467-76. doi: 10.1016/0092-8674(81)90388-3
[467 citations.]

Nº 3

Goldfarb, M.; Shimizu, K.; Perucho, M. and Wigler, M.: "Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells". *Nature*, 296 (Apr. 1982), 404-9. doi: 10.1038/296404a0
[469 citations.]

Nº 4

Pulciani, S.; Santos, E.; Lauver, A.V.; Long, L.K.; Robbins, K.C. and Barbacid, M.: "Oncogenes in human tumor cell lines: Molecular cloning of a transforming gene from human bladder carcinoma cells". *Proc Natl Acad Sci USA*, 79 (May. 1982), 2845-9. doi: 10.1073/pnas.79.9.2845

[256 citations. Research front, 83-1740, "Oncogenes and the genetics of human cancer: viral transforming genes and their DNA structure".]

Nº 5

Santos, E.; Tronick, S.R.; Aaronson, S.A.; Pulciani, S. and Barbacid, M.: "T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB-and Harvey- MSV transforming genes". *Nature*, 298 (Jul. 1982), 343-7. doi: 10.1038/298343a0

[532 citations. Research front, 83-1740, "Oncogenes and the genetics of human cancer: viral transforming genes and their DNA structure".]

Nº 6

Reddy, E.P.; Reynolds, R.K.; Santos, E. and Barbacid, M.: "A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene". *Nature*, 300 (Nov. 1982), 149-52. doi: 10.1038/300149a0

[1160 citations. Research front, 83-1740, "Oncogenes and the genetics of human cancer: viral transforming genes and their DNA structure".]

Nº 7

Pulciani, S.; Santos, E.; Lauver, A.V.; Long, L.K.; Aaronson, S.A.; Barbacid, M.: "Oncogenes in solid human-tumors". *Nature*, 300 (Dec. 1982), 539-42. doi: 10.1038/300539a0

[397 citations.]

Nº 8

Shimizu, K.; Goldfarb, M.; Perucho, M. and Wigler, M.: "Isolation and preliminary characterization of the transforming gene of a human neuroblastoma cell line". *Proc Natl Acad Sci USA*, 80 (Jan. 1983), 383-7. doi: 10.1073/pnas.80.2.383

[262 citations. Research front, 84-4046, "Characterization of human and murine cellular oncogene".]

Nº 9

Shimizu, K.; Goldfarb, M.; Suard, Y.; Perucho, M.; Li, Y.; Kamata, T.; Feramisco, J.; Stavnezer, E.; Fogh, J. and Wigler, M.H.: "Three human transforming genes are related to the viral ras oncogenes". *Proc Natl Acad Sci USA*, 80 (Apr. 1983), 2112-6. doi: 10.1073/pnas.80.8.2112

[313 citations. Research front, 84-4046, "Characterization of human and murine cellular oncogene".]

Nº 10

Santos, E.; Reddy, E.P.; Pulciani, S.; Feldmann, R.J. and Barbacid, M.: "Spontaneous activation of a human proto-oncogene". *Proc Natl Acad Sci USA*, 80 (Aug. 1983), 4679-83. doi: 10.1073/pnas.80.15.4679

[124 citations. Research article communicated by Severo Ochoa on 29 April 1983.]

Nº 11

Sukumar, S.; Notario, V.; Martín-Zanca, D. and Barbacid, M.: "Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of H-ras-1 locus by single point mutations". *Nature*, 306 (Dec. 1983), 658-61. doi: 10.1038/306658a0

[693 citations.]

Nº 12

- Guerrero, I.; Calzada, P.; Mayer, A. and Pellicer, A.: "A molecular approach to leukemogenesis: Mouse lymphomas contain an activated c-ras oncogene". *Proc Natl Acad Sci USA*, 81 (Jan. 1984), 202-5. doi: 10.1073/pnas.81.1.202
[205 citations.]
N° 13
- Nakano, H.; Yamamoto, F.; Neville, C.; Evans, D.; Mizuno, T. and Perucho, M.: "Isolation of transforming sequences of two human lung carcinomas: Structural and functional analysis of the activated c-K-ras oncogenes". *Proc Natl Acad Sci USA*, 81 (Jan. 1984), 71-5. doi: 10.1073/pnas.81.1.71
[130 citations.]
N° 14
- Santos, E.; Martín-Zanca, D.; Reddy, E.P.; Pierotti, M.A.; Della Porta, G. and Barbacid, M.: "Malignant activation of a K-ras oncogene in lung carcinoma but not in normal tissue of the same patient". *Science*, 223 (Feb. 1984), 661-4. doi: 10.1126/science.6695174
[298 citations.]
N° 15
- Lacal, J.C.; Santos, E.; Notario, V.; Barbacid, M.; Yamazaki, S.; Kung, H.F.; Seamans, C.; McAndrew, S. and Crowl, R.: "Expression of normal and transforming H-ras genes in Escherichia-Coli and purification of their encoded p21 proteins". *Proc Natl Acad Sci USA*, 81 (1 Sept. 1984), 5305-09. doi: 10.1073/pnas.81.17.5305
[111 citations.]
N° 16
- Guerrero, I.; Villasante, A.; D'Eustachio, P. and Pellicer, A.: "Isolation, characterization, and chromosome assignment of mouse N-ras gene from carcinogen-induced thymic lymphoma". *Science*, 225 (7 Sept. 1984), 1041-43. doi: 10.1126/science.6089339
[48 citations.]
N° 17
- Guerrero, I.; Villasante, A.; Corcés, V.; Pellicer, A.: "Activation of a c-K-ras oncogene by somatic mutation in mouse lymphomas induced by gamma radiation". *Science*, 225 (14 Sept. 1984), 1159-62. doi: 10.1126/science.6474169
[184 citations.]
N° 18
- Yamamoto, F. and Perucho, M.: "Activation of a human c-K-ras oncogene". *Nucleic Acid Res*, 12 (Dec. 1984), 8873-85. doi: 10.1093/nar/12.23.8873
N° 19
- Zarbl, H.; Sukumar, S.; Arthur, A.V.; Martin-Zanca, D. and Barbacid, M.: "Direct mutagenesis of Ha-ras-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats". *Nature*, 315 (May. 1985), 382-5. doi: 10.1038/315382a0
N° 20
- Pulciani, S.; Santos, E.; Long, L.K.; Sorrentino, V. and Barbacid, M.: "ras Gene amplification and malignant transformation". *Moll Cell Biol*, 5 (Oct. 1985), 2836-41.
[223 citations.]
N° 21
- Winter, E.; Yamamoto, F.; Almoguera, C. and Perucho, M.: "A method to detect and characterize point mutations in transcribed genes: Amplification and overexpression of the mutant c-Ki-ras allele in human tumor cells". *Proc Natl Acad Sci USA*, 82 (Nov. 1985), 7575-9. doi: 10.1073/pnas.82.22.7575
[356 citations. *Research article communicated by Severo Ochoa on 25 July 1985.*]
N° 22
- Guerrero, I.; Villasante, A.; Corces, V. and Pellicer, A.: "Loss of the normal N-ras allele in a mouse thymic lymphoma induced by a chemical carcinogen". *Proc Natl Acad Sci USA*, 82 (Dec. 1985), 7810-4. doi: 10.1073/pnas.82.23.7810
[117 citations.]
N° 23
- Winter, E. and Perucho, M.: "Oncogene amplification during tumorigenesis of established rat fibroblasts reversibly transformed by activated human ras oncogenes". *Moll Cell Biol*, 6 (Jul. 1986), 2562-70.
[51 citations.]
N° 24
- Jordano, J. and Perucho, M.: "Chromatin structure of the promoter region of the human c-K-ras gene". *Nucleic Acid Res*, 14 (Sept. 1986), 7361-78. doi: 10.1093/nar/14.18.7361
[22 citations.]
N° 25
- Guerrero, I.; Wong, H.; Pellicer, A. and Burstein, D.E.: "Activated N-ras gene induces neuronal differentiation of PC12 rat pheochromocytoma cells". *J Cell Physiol*, 129 (Oct. 1986), 71-6. doi: 10.1002/jcp.1041290111
[146 citations.]