TIMELINE

Retroviral oncogenes: a historical primer

Peter K. Vogt

Abstract | Retroviruses are the original source of oncogenes. The discovery and characterization of these genes was made possible by the introduction of quantitative cell biological and molecular techniques for the study of tumour viruses. Key features of all retroviral oncogenes were first identified in *src*, the oncogene of Rous sarcoma virus. These include non-involvement in viral replication, coding for a single protein and cellular origin. The *MYC*, *RAS* and *ERBB* oncogenes quickly followed *SRC*, and these together with *PI3K* are now recognized as crucial driving forces in human cancer.

This article is dedicated to Harry Rubin. His pioneering work started the field.

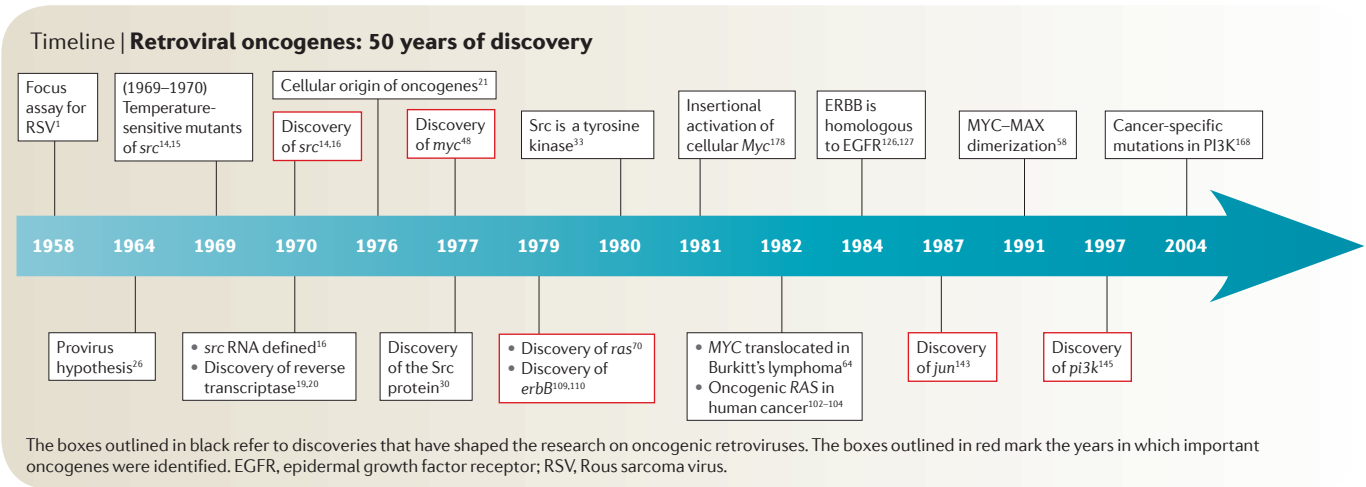
Most oncogenes that have predominant roles in human cancer were first recognized in retroviruses. This includes the receptor tyrosine kinase epidermal growth factor receptor (EGFR), the small GTPase RAS, the phosphoinositide 3-kinase PI3K and the transcriptional regulator MYC. The discovery of retroviral oncogenes during the past four decades has set in motion an era of progress that has culminated in our current view of cancer as a genetic disease (TIMELINE) — a view that guides and inspires therapeutic innovations. It currently seems to be attractive to look back to the origins of the oncogene field, as they illustrate the first principles that are still valid and applicable to the legions of oncogenes encountered today.

There are slightly more than 30 retroviral oncogenes, which have been identified almost exclusively in avian and rodent viruses. Their products can be grouped into eight functional classes (TABLE 1). The unifying functional assignment of these genes and proteins is signalling in the control of cellular replication. From this list, I discuss a few oncogenes that best illustrate the history of experimental and theoretical breakthroughs but that also have crucial roles in human disease.

The *src* paradigm

The first retroviral oncogene to be discovered was *src*: this was no accident. Preparations of Rous sarcoma virus (RSV), the avian sarcoma virus that carries the *src* gene, induce readily visible oncogenic transformation within a few days in primary fibroblasts. RSV can be accurately titrated in cell culture, with a focus assay developed in 1958 (REF. 1). In this assay, the focus number is directly proportional to the amount of virus, hence a single RSV particle can fully transform a host cell, and no cooperation between complementing viruses is required. Soon, methods for the biological cloning of RSV particles were developed, which were the fruit of extensive studies devoted to a replication-defective variant of RSV[2,3]. A procedure for assaying non-oncogenic but actively replicating avian retroviruses by interference with RSV focus formation was also devised[4]. In the 1960s, these were powerful quantitative cell biological tools, and the avian sarcoma viruses were the only retroviruses for which such tools were available. This technological advantage was decisive in the discovery of the first oncogene.

Our knowledge of *src* and of its protein product is the culmination of a long and complex evolution with stepwise, successive contributions from genetics, biochemistry,



The boxes outlined in black refer to discoveries that have shaped the research on oncogenic retroviruses. The boxes outlined in red mark the years in which important oncogenes were identified. EGFR, epidermal growth factor receptor; RSV, Rous sarcoma virus.

immunology and structural biology[5]. Each of these steps built on and complemented the preceding one. Three early genetic observations helped to define the problem: first, there are mutants of RSV that, instead of transforming the fibroblast host into a rounded cell, induce an elongated fusiform cell shape[6,7]. Therefore, the phenotype of the transformed cell is under the control of the viral genome. Second, a replication-defective variant of RSV transforms cells without producing infectious progeny, which indicated that the generation of progeny virus is not a prerequisite for oncogenicity[8,9]. Third, most strains of RSV are non-defective[10,11] (meaning that they carry all viral-replicative genes and the oncogene in the same RNA molecule) (FIG. 1), but they spontaneously segregate deletion mutants that still replicate but can no longer transform cells[12,13]. Reproduction and oncogenicity are separate and distinct functions.

The proof for the existence of a viral gene that initiates and maintains the transformed cellular phenotype came from experiments with temperature-sensitive mutants. In 1970, a groundbreaking paper in *Nature* described a mutant of the replication-competent Schmidt–Ruppin strain of RSV that transforms cells at a low, permissive temperature but that fails to transform cells at an elevated, non-permissive temperature[14]. However, the production of progeny virus is unaffected by temperature. This mutant pointed to the existence of a viral gene that directs oncogenicity but that is dispensable for virus replication. An earlier report of temperature-sensitive mutants of RSV had also demonstrated this temperature dependence of transformation, but the temperature effect also extended to virus replication, probably owing to multiple mutations[15].

Biochemistry then provided the physical underpinning for the existence of a specific oncogene in RSV. This work depended on a unique property of the RSV genome: its non-defectiveness (as discussed above). All other oncogene-carrying retroviruses are replication defective, the oncogene having displaced one or several of the viral-replicative genes. Mutant RSVs that are transformation defective, but that are replication competent, contain a smaller RNA than the parental virus, suggesting that the lost sequences represent the oncogene[16] (FIG. 1). This hypothesis was supported by genetic mapping experiments. Temperature-sensitive mutations that affect the ability to transform cells were located to the region of the RSV genome that is deleted in the transformation-defective viruses[17]. Biochemical mapping with RNA fingerprinting showed that the deleted RNA was a contiguous fragment, located at the 3′ terminus of the viral RNA genome[18]. Thus, this was a piece of the retroviral genome that was not required for virus survival but that was essential for oncogenic transformation. The fact that this gene was readily lost from the viral genome showed that it did not convey an evolutionary advantage to the virus.

Table 1 | **Functional classes of retroviral oncoproteins**

Functional class	Examples	Source virus
Growth factor	Sis (PDGFB)	Simian sarcoma virus
Receptor tyrosine kinase	ErbB (EGFR)	Avian erythroblastosis virus
Hormone receptor	ErbA (THRA)	Avian erythroblastosis virus
G protein	Ha-ras, a GTPase	Harvey sarcoma virus
	Ki-ras, a GTPase	Kirsten sarcoma virus
Adaptor protein	Crk, a modular signalling link	CT10 avian sarcoma virus
Non-receptor tyrosine kinase	Src, a signalling protein kinase	Rous sarcoma virus
	Abl, a signalling protein kinase	Abelson murine leukemia virus
Serine/threonine kinase	Akt, a signalling protein kinase	Akt8 murine thymoma virus
	Mos, a signalling protein kinase	Moloney murine sarcoma virus
Transcriptional regulator	Jun, a component of the AP1 complex	Avian sarcoma virus 17
	Fos, a component of the AP1 complex	Finkel–Biskis–Jinkins murine sarcoma virus
	Myc, a transcription factor	Avian myelocytomatosis virus MC29
Lipid kinase	Pi3k	Avian sarcoma virus 16

AP1, activator protein 1; EGFR, epidermal growth factor receptor; Ha-ras, Harvey-ras; Ki-ras, Kirsten-ras; PDGFB, platelet-derived growth factor-β; THRA, thyroid hormone receptor-α.

Where did this accessory piece of information come from? The biochemical experiments had defined a distinct nucleic acid segment of the retroviral RNA genome as the oncogene, and this definition then paved the way for the physical isolation of *src*. The discovery of reverse transcriptase in 1970 shifted the biochemistry of retroviruses from RNA to DNA, for which there existed better and more versatile tools of experimentation[19,20]. One of these tools, subtractive hybridization, was applied to DNA transcripts of non-defective RSV and its replication-defective deletion mutant and so resulted in the isolation of *src*-specific DNA sequences. With these sequences it was possible to explore the origin of *src*, using hybridization as a measure of relatedness. These experiments showed that *src* originated from the cellular genome and that it was a cellular, not a viral, gene[21]. This fundamental insight, at first ridiculed, was soon extended to other retroviral oncogenes that had been discovered in the meantime, and it changed the landscape of tumour virology[22]. Retroviruses were no longer originators of oncogenic information: they were demoted to mere carriers of oncogenes that are part of the host genome. This discovery resulted in a huge expansion of the oncogene concept. Any cellular gene with an oncogenic potential that could be activated by a gain of function qualified as an oncogene. Most of these activating genetic events do not involve viruses, but retroviruses that lack an oncogene in their genome can still activate cellular oncogenes by insertional mutagenesis (BOX 1).

The essential foundation for the genetics of *src* and of other retroviral oncogenes is the unique life cycle of retroviruses, which involves reverse transcription of the virion RNA into DNA, and the integration of this DNA into the host genome[23–25]. The genetic stability of the oncogenic phenotype induced by RSV had prompted Temin[26,27] to propose the main elements of such a life cycle as the 'provirus hypothesis'. At the time, this seemed a preposterous idea because RNA-dependent synthesis of DNA overturned the central dogma of unidirectional flow of genetic information from DNA to RNA to protein. The sensitivity of retrovirus replication to inhibitors of DNA synthesis supported Temin's claim, but the evidence was far from compelling until the discovery of reverse transcriptase provided firm proof for the provirus hypothesis[19,20]. Today, reverse transcriptase is used as a routine tool for copying genetic information, so it is important to remember that the generation of a double-stranded DNA copy from virion RNA and the integration

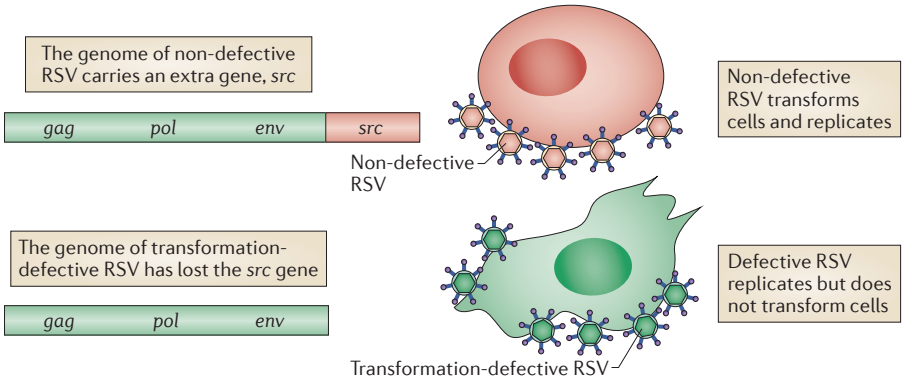


Figure 1 | **The biochemical definition of v-*src*.** The protein-coding regions of non-defective Rous sarcoma virus (RSV) encompass the complete information required for virus reproduction (*gag*, *pol* and *env*) and the information needed for oncogenic transformation (*src*). The RSV-infected cell produces progeny virus and is transformed. During the replication of RSV, mutant viruses are generated that are no longer oncogenic but that contain all the essential viral genes and are fully capable of producing progeny virus that fails to transform cells in culture. A comparison of the genome sizes of parental RSV and the transformation-defective mutant shows that loss of oncogenicity is correlated with loss of about 20% of the genome. The lost sequences represent the *src* gene, which is not essential for virus replication. Using DNA transcripts of these two viral genomes, *src* sequences can be purified by subtractive hybridization.

of the provirus into the cellular genome are at the root of our understanding of retroviral oncogenes. Proviral integrations are genetic recombination events that can result in the incorporation of a cellular oncogene into the viral genome (FIG. 2). Such acquisitions are rare; they can occur during viral passage in an animal but they are almost never seen in cell culture. There is no experimental system that predictably reproduces spontaneous oncogene acquisition; therefore, the molecular details of this process remain hypothetical[28,29].

The data on the *src* gene had left an important question unanswered: what is the product of this oncogene? Considering the technical arsenal available at the time, it was not an easy question to answer. A phenomenal breakthrough was achieved in 1977 with a v-Src-specific antibody raised by a technique that was as ingenious as it was not obvious: injecting a mammalian-adapted RSV into young rabbits[30]. This antibody identified the v-*src* product as a 60 kDa protein that was soon found to have protein kinase activity[31,32]. The crucial insight that differentiated the SRC kinase from other protein kinases known at the time came with the discovery of its target amino acid: it is not serine or threonine, but tyrosine[33]. The SRC protein was the first representative of this new class of tyrosine protein kinases, rapidly followed by EGFR[34]. Today, the members of this class are actively studied, and they have key regulatory functions in the cell[35].

In the early 1980s, the cellular *SRC* and *v-src* genes were sequenced[36–39]. The viral Src protein differs from its cellular progenitor by a carboxy-terminal deletion, which includes a crucial regulatory phosphorylation site, and by several point mutations. A comparison of the two proteins showed that the cellular SRC had a lower kinase and negligible oncogenic activity compared with viral Src[40–42]. The explanation of this difference evolved from the discovery that cellular SRC carries two modular protein–protein interaction domains, a phosphotyrosine-binding SH2 domain and a poly-proline-binding SH3 domain[43,44]. Both are crucial for the regulation of SRC kinase activity. The molecular details of this regulation were revealed by the crystal structure of SRC and of the SRC family kinase HCK[45,46]. Cellular SRC requires activation that opens the catalytic domain by disrupting intramolecular interactions involving both the SH2 and the SH3 domains. In viral Src, these inhibitory interactions are absent because of the C-terminal deletion and point mutations in the SH3 domain, making viral Src constitutively active.

The kinase activity of SRC invited a search for target proteins that would shed light on the normal and oncogenic functions of the enzyme. Multiple direct and indirect SRC targets have been identified, but the search for cancer-relevant functions is far from complete and remains an active area of cancer research[47].

Discovering diversity

For the discovery and characterization of other retroviral oncogenes, some lessons from *src* could be transferred, but there were also new and unique challenges to overcome. Other retroviruses that carry an oncogene are replication defective, in contrast to non-defective RSV. Replication-defective viruses require a helper virus that supplies the missing viral functions in *trans*. These viruses always occur as mixtures of transforming virus and non-transforming helper virus. Because of this dependence on a helper, the genetic experiments are less straightforward than with RSV. However, the structure of the genomes of replication-defective viruses can also offer an advantage: the displacement of viral-replicative genes by an oncogene can generate a fusion gene, combining cell-derived and viral sequences, and resulting in the production of an oncogenic fusion protein. Such viral–cellular fusion products are readily identifiable with available viral antibodies.

A standard succession of events characterizes the history of most retroviral oncogenes. It starts with the identification of the gene in the virus. Here, two criteria that were first established for *src* have become signature traits of almost all retroviral oncogenes: cellular origin and non-identity with viral-replicative genes. Identification of the protein, cloning and sequencing are the next steps, and are extended to the cellular counterpart of the gene. Questions of oncogenic and normal functions are then addressed, with such studies building on pre-existing knowledge of the cellular protein. In the early days of oncogene discovery, temperature-sensitive mutants had an important role. With the advances in cloning and sequencing, identifying such mutants became less important. The discovery of oncogenes in DNA viruses also started with temperature-sensitive mutants[31]. However, the genetic origins and molecular mechanisms of these oncogenes and oncoproteins stand in contrast to those of retroviruses. The crucial differences are summarized in BOX 2.

The potent trio in human cancer

MYC. One of the first oncogenes that emerged after *SRC* was *MYC*. An RNA fingerprint analysis of the genome of the avian myelocytomatosis virus MC29 had revealed oligonucleotides that were unrelated to viral-replicative genes and to *src*[48]. The same sequences were also identified in the avian retroviruses CMII, OK10 and MH2 (REFS 49–51). The sequences were not scattered over the genome but were shown to form a contiguous stretch of RNA, indicating that they were derived from a distinct gene. A fusion protein combining viral Gag sequences of MC29 with the presumptive new oncoprotein was rapidly identified with viral antibodies[52]. DNA

Box 1 | **Activation of cellular oncogenes by insertional mutagenesis**

Retroviruses of the subfamily oncovirus that lack an oncogene in their genome are able to induce cancer by insertional mutagenesis[178]. In this process, a provirus integrating in the vicinity of a cellular oncogene functions as a positive transcriptional regulator and thus activates the latent tumorigenic potential of the cellular gene. Insertional, retrovirus-mediated mutagenesis is a slow process that occurs only in the vertebrate host and that typically requires prolonged and extensive viral replication and integration. It has been widely used to reveal the oncogenic potential of cellular genes that are never transduced by viruses[179].

sequencing had just been invented[53,54], and within a few years it was applied to the viral *myc* and the human *MYC* genes[55,56].

A first important insight into the functions of the MYC protein came with the discovery that it is localized in the cell nucleus[57]. One of the possible roles for this protein was to act as a transcriptional regulator. However, the failure of MYC to bind DNA under physiological conditions could not be easily reconciled with this idea. This impasse was broken with the discovery of the MAX protein as an obligatory dimerization partner of cellular MYC. Only the MYC–MAX heterodimer can bind DNA with a high affinity and affect transcription[58]. MAX is the required partner of several MYC-related proteins, forming the central component of a regulatory network that can stimulate, as well as repress, transcription[59]. The workings of this network are based on selective dimerization. MAX forms DNA-binding homodimers, but none of its partners has this ability, so they depend on dimerization with MAX to bind DNA and to regulate transcription.

The identification of MYC-target genes has been challenging because thousands of copies of its short DNA target sequence, the E-box CACGTG, are present in vertebrate genomes. A recent study using a combination of chromatin immunoprecipitation and deep sequencing identified more than 7,000 genomic-binding sites in a cell that overexpresses MYC[60]. Cellular levels of MYC are tightly regulated, and overexpression leads to uncontrolled cell replication or to apoptosis, depending on contextual factors that are not yet completely understood.

There are three *MYC* genes in the human genome: c-*MYC* (also known as *MYC*), *MYCL1* and *MYCN*[55,61,62]. The cellular homologue of the retroviral *myc* gene is c-*MYC*. *MYCN* and *MYCL1* were discovered later in human cells, and they have important roles in diverse human cancers[63]. The two representative mechanisms for the involvement of *MYC* in human disease came to light from studies of Burkitt's lymphoma and neuroblastoma. Burkitt's lymphoma cells always carry a chromosomal translocation that places c-*MYC* under the control of an immunoglobulin enhancer[64]. The result is increased transcription of c-*MYC* driven by the immunoglobulin-regulatory sequences. The discovery of c-*MYC* rearrangements in a human lymphoma was the first indication that cellular counterparts of retroviral oncogenes are involved in the pathogenesis of human disease. In neuroblastoma, the *MYCN* gene is frequently amplified, and the expression of MYCN is correspondingly elevated[65]. Upregulated transcription and amplification are the two mechanisms for the oncogenic gain of function in the *MYC* genes. Mutations in the coding region of *MYC* do not have an important role in human cancer.

Recent studies indicate that the role of *MYC* in cancer goes beyond the situations in which it seems to be the primary driver. c-*MYC* has emerged as the mediator of resistance to inhibitors of PI3K, and dominant-negative MYC causes regression of RAS-induced tumours in mice[66,67].

RAS. The isolation of v-*ras* presented a different set of challenges. The two principal viruses carrying this oncogene, Harvey sarcoma virus and Kirsten sarcoma virus, arose by recombination with the host genome during passage of murine leukaemia virus in rats[68,69]. The rat-derived oncogene in these replication-defective viruses is not fused to viral genes, and in the absence of such viral markers, the Ras protein could not be identified with viral antibodies. However, animals bearing Kirsten or Harvey sarcomas generated antibodies that interacted with the 21 kDa product of the *ras* gene[70]. The Ras protein was also obtained by *in vitro* translation of the viral genome[71]. Cloning and sequencing of the Harvey and Kirsten sarcoma viruses defined the viral and rat-derived contributions to these recombinant genomes and completed our molecular knowledge of the viral *ras* gene[72–74]. The two *ras* genes, Kirsten-*ras* (Ki-*ras*) and Harvey-*ras* (Ha-*ras*), do not differ in the properties we consider here.

A first clue about biochemical functions came from the observation that RAS has guanine nucleotide-binding activity, a finding that quickly culminated in the discovery that RAS is a GTPase[75–78]. In its active form, RAS is bound to GTP, and binding could be enhanced by activated EGFR[79]. How could RAS be integrated into cellular signalling, and what was responsible for its oncogenic activity? Part of the answer came from linking an adaptor protein and a guanine nucleotide exchange factor to the activity of RAS[80,81]. The SH2 domain of the adaptor, GRB2, binds to phosphorylated tyrosine, typically in a receptor tyrosine kinase (such as EGFR), and with its SH3 domain GRB2 can recruit the guanine nucleotide exchange factor (GEF) SOS. SOS stimulates the release of GDP from RAS and thus enhances loading with GTP. This sequence of interactions established the upstream signalling path that leads to RAS activation[82].

The other part of the answer, outlining the downstream activities of RAS, was initiated by the discovery of the *raf* oncogene in a murine sarcoma virus[83,84] and of its avian homologue *mil* in the chicken tumour virus MH2 (REFS 85,86). The RAF protein binds to GTP-loaded RAS and connects it to the MAPK pathway[87–89]. Activated RAS also binds to the catalytic subunit of PI3K, and this interaction is important for PI3K signalling[90]. Although numerous somatic mutations occur in the catalytic subunit of human PI3K, no mutations have been found in the RAS-binding domain, suggesting that interaction with RAS is essential for the function of PI3K. The oncogenic activities of PI3K are discussed in greater detail below.

The GTPase activity of RAS is stimulated by association with a GTPase-activating protein (GAP)[91]. RAS acquires oncogenic potency by point mutations affecting residues 12 and 61. These mutations disturb the interaction with GAPs. They reduce the rate of GTP hydrolysis and result in elevated levels of the active, GTP-bound Ras[78,91–96]. An important aspect of all RAS activity is cellular localization. RAS is positioned at the inner side of the plasma membrane, and this location is essential for its activity[97]. The interaction with membrane lipids is mediated by an obligatory post-translational isoprenylation of the protein[98,99].

A series of exciting and dramatic experiments directly linked RAS to human cancer. Initially, the transfer of DNA from human cancer cells was found to transform recipient mouse cells. Integration of the source DNA into the genome of the recipient cells was verified by the presence of readily identifiable repetitive human sequences[100,101]. This breakthrough came with the discovery that the transforming DNA that was derived from human cancer cells is homologous to *ras*[102–105]. This discovery also linked a retroviral oncogene that in experimental systems induces

sarcomas to epithelial cancers in humans. The activity of retroviral oncogenes is clearly not restricted to fibroblasts or haematopoietic cells but also includes epithelial cells. Oncogenic activity in DNA transfection experiments also revealed the existence of a third *RAS* gene, *NRAS*, in the human genome[106].

In the span of 2 years, 1982 to 1984, the findings of c-*MYC* in Burkitt's lymphoma, *MYCN* in neuroblastoma and oncogenic *RAS* in diverse human cancers linked the two retroviral oncogenes unequivocally to human disease as probable causative agents. These connections between retroviral model systems and human cancer could have been predicted from the cellular origin of retroviral oncogenes, but they came as a surprise nonetheless. The discoveries of *MYC* and *RAS* have special historical importance because they have consolidated the view of cancer as a genetic disease.

ERBB. The story of *erbB* takes us back to the early days of retrovirology. *erbB* is the oncogene of avian retroviruses that induce an acute form of erythroid leukaemia called erythroblastosis.The discovery of one of these viruses, referred to as strain R, dates from 1935 (REF. 107). It contains two cell-derived oncogenes, *erbA,* a hormone receptor, and *erbB*[22,108–110]. For the induction of oncogenic growth, *erbA* is auxiliary but dispensable, whereas *erbB* is both necessary and sufficient, as a separate isolate of erythroblastosis virus, strain H, carries only the *erbB* oncogene but does not differ substantially from strain R in tumour spectrum or pathogenic potency[111]. Studies on additional independent isolates of avian erythroblastosis virus have supported this dominant role of *erbB* in oncogenesis[112]. Analyses of the cloned genomes and of *in vitro* translated proteins from strain R and H viruses suggested that the viral ErbB protein is produced by a fused mRNA consisting of a very short amino-terminal viral sequence and part of the cellular *ERBB*[113–116]. In addition, specific antibodies detected a 74 kDa transformation-specific protein in cells infected by avian erythroblastosis virus[117,118].

At the time, this information on chicken viruses seemed almost esoteric, but it has acquired great relevance for human disease. In a period of a few months in 1984, the viral ErbB protein was found to be glycosylated and phosphorylated, as well as structurally related to tyrosine kinases. It showed sequence features of tyrosine kinases and was localized as an integral membrane protein at the cell surface[115,119–125]. Finally, and most importantly, sequence analysis revealed both close homology to cellular EGFR and a large deletion in the extracellular domain of viral ErbB[126,127]. In addition to the N-terminal truncation, viral ErbB proteins show mutations in the kinase domain located in the C-terminal cytoplasmic portion of the protein. Viral ErbB functions as a constitutively active receptor tyrosine kinase; the activity is ligand-independent and also requires the kinase domain mutations. The mutations in viral ErbB do not merely cause a quantitative enhancement of the same signalling pathways that are controlled by cellular EGFR: they induce qualitative changes in the spectrum of signalling targets. These changes are crucial to the oncogenic potency of the protein[112].

EGFR can function as an oncogenic 'driver' in diverse human cancers. Mutations that mechanistically resemble those seen in viral ErbB occur in EGFR in glioblastoma and non-small-cell lung cancer. About 50% of glioblastomas carry the EGFRvIII mutant, which has lost a large portion of the extracellular domain and which no longer binds ligand but signals constitutively, addressing targets that are different from those of wild-type EGFR[128]. Such a cancer-specific mutation in a kinase would seem to be an ideal therapeutic target. However, the clinical experience with inhibitors of EGFR in glioblastoma has been uneven, with tumour shrinkage linked to the co-expression of EGFRvIII and the tumour suppressor PTEN[129]. In non-small-cell lung cancer, EGFR mutations are located in the kinase domain and lead to constitutive autophosphorylation and activation. Such cancers, seen mostly in non-smokers, are uniquely sensitive to EGFR inhibitors but regularly develop resistance to these drugs[130–133].

The human genome contains three additional genes that are closely related to *EGFR*: *HER2*, *HER3* and *HER4* (also known as *ERBB2*, *ERBB3* and *ERBB4*, respectively)[134–136]. The oncogenic potential of *HER2* was discovered in transfection experiments with DNA from human neuroblastoma cells. The cell-transforming gene in these experiments was identified as *EGFR*-related, with an activating mutation in the transmembrane domain[137]. *HER2* is frequently amplified in breast cancer[138]. A humanized monoclonal antibody that inhibits HER2 signalling (trastuzumab) shows substantial clinical benefit and is now part of standard therapy for *HER2*$^+$ breast cancers[139]. *HER3* is unusual in that it has extremely low kinase activity and functions predominantly as a dimerization partner of other EGFR family members[140]. *HER4* differs from the other EGFR-related genes in that it mediates cellular differentiation and inhibits replication[141].

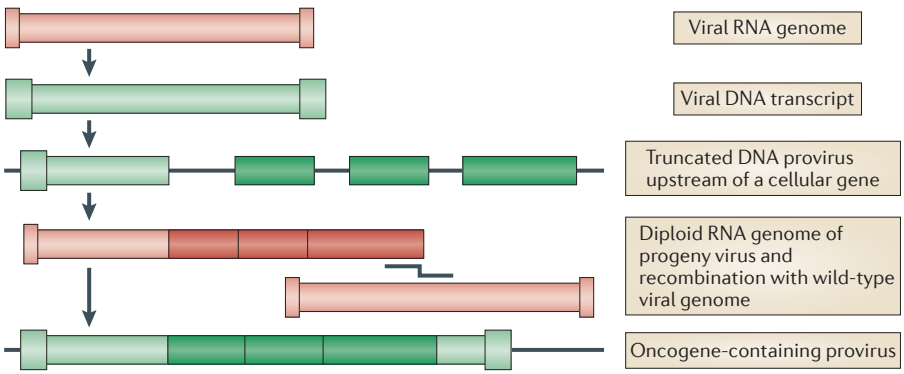


Figure 2 | **Acquisition of a cellular oncogene by a retroviral genome.** First, virion RNA is transcribed into double-stranded DNA (RNA is shown in red and DNA is shown in green). Second, an accidentally truncated provirus is located upstream of a cellular gene (cellular exons are indicated in dark green; lighter colours represent viral sequences, and darker colours represent cellular sequences). Third, a spliced fusion transcript of viral and cellular sequences is packaged into a progeny virion together with a wild-type viral genome (retroviruses are diploid). Finally, during next-generation reverse transcription, recombination between the two genomes generates a DNA provirus composed of the cellular-oncogene-encoding sequence fused to viral sequences. As a result of acquiring cellular sequences, viral information that is essential for the production of progeny is lost, and such highly oncogenic viruses are replication defective, with the exception of most strains of Rous sarcoma virus (RSV), which can reproduce and are oncogenic. This mechanism for the acquisition of cellular sequences is hypothetical but in agreement with available experimental data.

Oncogenes from slaughterhouse viruses

In the 1980s, it became clear that avian retroviruses are a particularly rich source of oncogenes. In chickens, retrovirus infection is common and widespread. Most of these viruses are replication-competent, do not carry an oncogene and induce tumours (mostly lymphoid leukosis) by insertional activation of a cellular oncogene[142]. But occasionally, the genetic recombination between

Box 2 | **Contrasting mechanisms in viral oncogenicity: RNA versus DNA viruses**

The oncogenes of retroviruses are cell-derived; they deregulate cellular signalling and transcriptional controls. By contrast, oncogenic DNA viruses, including the papilloma viruses, polyoma virus, simian virus 40 (SV40) and some tumour-inducing adenoviruses and herpesviruses, carry their own oncogenes. Some of the best understood among these disrupt the RB protein-mediated control of the cell cycle[180–184]. During the G1 phase of the cell cycle, the RB protein is hypophosphorylated and bound to E2F transcription factors, forming transcriptional repressor complexes. These are essential components of the restriction point that prevents entry into the S phase of the cell cycle. On mitogenic stimulation, cyclin-dependent kinases phosphorylate RB, thus releasing the E2F proteins, which then initiate a transcriptional programme that marks the entry into the S phase. Several DNA viral proteins bind to hypophosphorylated RB: the E1A protein of adenoviruses, the large T antigen of SV40 virus and the E7 proteins of oncogenic human papilloma viruses. These interactions free the E2F proteins without a requirement for mitogenic signals and start the S phase of the cell cycle. Proteins of oncogenic DNA viruses can also operate as constitutive signalling receptors or can interfere with the functions of inhibitors of cyclin-dependent kinases[185].

virus and host can result in the incorporation of an oncogene into the viral genome. Such an acquisition converts the virus from slowly oncogenic to rapidly oncogenic, resulting in solid tumours that are distinct from endemic leukosis (FIG. 2). Chicken slaughterhouses process up to 30,000 birds a day, and each of these chickens is inspected for signs of disease. At these numbers, even rare viral–cellular recombination events that result in aggressive cancers can be found.

Slaughterhouse veterinarians have greatly facilitated the discovery of several new, rapidly oncogenic retroviruses, and from these viruses, three new oncogenes were isolated: *jun, qin* and *pi3k*[143–145]. The discovery of the retroviral Jun, the finding of its cellular counterpart in the transcription factor complex activator protein 1 (AP1), and the identification of the tight partnership with the oncoprotein Fos (discovered separately in the Finkel–Biskis–Jinkins murine sarcoma virus[146]), marked an exciting period in the history of oncogenes. The story of these events has been told elsewhere[147,148]. QIN (also known as FOXG1) is a representative of the winged helix or FOX family of DNA-binding proteins that function as developmental and metabolic transcriptional regulators[144]. Although QIN has not been implicated in human cancer, the FOX protein family is linked to human disease by the involvement of *FOXO1* in a chromosomal translocation that contributes to the development of alveolar rhabdomyosarcoma, which is an aggressive childhood tumour. Another member of the family, FOXA1, controls the sexual dimorphism seen with hepatocellular carcinoma[149]. A broader survey of the association of FOX proteins with cancer has been presented in a recent review[150].

Among the oncogenes derived from these recently isolated avian retroviruses, *pi3k* stands out because its cellular counterpart controls signalling pathways that show aberrant activation in most human tumours and also contain promising drug targets. Retroviral *pi3k* has served as an important model for the oncogenic activities of human PI3K. There has long been a suspicion that the lipid kinase PI3K may have oncogenic potential. In early work, the oncoproteins of DNA viruses, as well as Src, were shown to be associated with a cellular lipid kinase activity[151–155]. This interaction was essential for the oncogenicity of these viral proteins. The transformation-associated lipid kinase activity was then found to catalyse the phosphorylation of the D3 position of the inositol ring, defining a novel enzymatic activity that generates phosphatidylinositol-3-phosphates[156]. The fundamental importance of this finding was not realized until much later when it became clear that this PI3K was at the centre of an extensive and versatile cellular signalling network that becomes corrupted in most cancers[157,158]. Direct evidence for the oncogenicity of PI3K came with the discovery of an avian haemangiosarcoma virus, ASV16, in a tumour obtained from a chicken-processing plant. ASV16 is a replication-defective virus with a genome that encodes a single protein encompassing the p110α isoform of the catalytic subunit of chicken PI3K fused N-terminally to viral *gag* sequences[145].

Viral *pi3k* harbours several mutations in the p110α-coding sequence, but these do not induce a gain of function and are irrelevant for oncogenic activity. Oncogenicity depends on the N-terminal Gag sequences[159]. The Gag sequences were initially thought to facilitate membrane localization and bring the enzyme in direct contact with its substrate. Support for this idea comes from the observation that a myristylation signal added to the N terminus of cellular p110α also has a strongly activating effect and makes the protein oncogenic. However, recent data have cast doubt on this interpretation. Even random amino acid sequences, added to the N terminus of p110α, are activating, and there is no requirement for a membrane-localizing function in these sequences. Rather, these N-terminal additions seem to induce a conformational change that mimics the activation of p110α by upstream signals[160]. A similar mechanism for constitutive activity is seen with cancer-specific mutations that carry an amino acid substitution in the helical domain of p110α[161–164]. In such mutants, the inhibitory interaction with the regulatory subunit p85 is disrupted.

PI3K has moved into the limelight as a cancer target because of frequent cancer-specific genetic and epigenetic changes that result in enhanced activity. These include loss-of-function mutations in the PI3K antagonist and tumour suppressor PTEN, increased activity and amplification of PI3K, and gain-of-function mutations in the catalytic subunit p110α[165–170]. Enhanced PI3K signalling is a driving force in cancer development. Academic laboratories and the pharmaceutical industry have responded to this situation by generating small-molecule inhibitors of PI3K, and several of these are currently in advanced clinical trials[171].

From simplicity to complexity

As we look at the history of oncogenes and their importance in human disease, two developmental trends unfold (TIMELINE). One is a steady increase in relevance, the other a broadening of the concept of cancer as a genetic disease. Rapidly tumorigenic retroviruses that carry oncogenes have mostly been found in chickens and in mice. Early work with these viruses focused on cancer as an infectious condition. But the concepts and mechanisms uncovered with readily transmissible animal tumours did not seem to be applicable to the human situation. Therefore, the importance of identifying specific oncogenes in viruses was at first exclusively experimental and theoretical. These discoveries showed that normal vertebrate cells could be transformed into cancer cells by the action of a single gene. This was a revolutionary insight, offering simplicity and the prospect of complete molecular understanding.

Retroviral oncogenes remained mainly experimental tools with uncertain ties to human cancer until 1976, when oncogene sequences were found in cellular genomes[21]. This discovery transformed the field. Retroviruses, with their ability to acquire and transduce host genes, became just one of several possible ways by which a cellular oncogene can be activated. In principle, any genetic change in the cellular oncogene is

Table 2 | **Oncogenes first identified in retroviruses as drivers in human cancer**

Oncogene	Mechanism of activation	Cancer type	Refs
MYC	Increased transcription	Burkitt's lymphoma	64,186
	Increased transcription	B cell lymphoma	187,188
	Amplification	Neuroblastoma	65,189
	Amplification	Medulloblastoma	190–192
EGFR	Mutation	Glioblastoma	128,193
	Mutation	Non-small-cell lung cancer	130–133
RAS	Mutation	Pancreatic cancer	194–196
RAF	Mutation	Melanoma	197

EGFR, epidermal growth factor receptor.

potentially activating. The next transformative step on the way to relevance established the direct connection between the cellular versions of retroviral oncogenes and human cancer. The key breakthroughs were finding transcriptional activation of c-*MYC* by chromosomal translocation in Burkitt's lymphoma, detecting amplification of *MYCN* in neuroblastoma and identifying activated *RAS* in DNA from human cancer cells[64,65,102–104]. These and other human cancer-driving retroviral oncogenes are listed in TABLE 2.These findings were fundamental in revealing cancer as a genetic disease. They also seemed to be a reductionist triumph, explaining cancer with changes in one, or at the most a few, genes that would generate novel and highly specific therapeutic targets.

This development took retroviral oncogenes from obscurity to prominence, but in subsequent years, genetic changes that affect the oncogenic cellular phenotype have steadily increased in type and in number. If we define an oncogene as a replication-promoting gene that encodes a protein and shows gain of function in cancer, then the number of such genes is probably in excess of 1,000 and growing. A comprehensive view of cell-autonomous genetic alterations in cancer further includes tumour suppressors that contribute to the oncogenic phenotype by a loss of function, often as a result of epigenetic changes[172]. MicroRNAs have added another layer of complexity, with both pro-oncogenic and anti-oncogenic effects[173]. The vast extent of the non-coding transcriptome, including large antisense transcripts and pseudogenes, is beginning to be functionally explored and probably holds even more surprises[174]. Cancer genome projects have uncovered an unexpected multitude of genetic changes in all cancers, revealing mutational landscapes that are characteristic of tumour origin and histology. A similar trend towards complexity can be seen in our understanding of oncoprotein functions. All these proteins show multiple activities, generating diverse signals. A complete molecular understanding of how these activities initiate and maintain cancer remains a challenge.

The complexity of genetic alterations becomes irrelevant in certain cancers that show a striking and apparently irreversible dependency on a single, dominant genetic change. Such oncogene addiction can be the basis for stunning clinical successes with targeted therapy[175]. However, it is questionable whether the model of cellular addiction to a single oncoprotein is applicable to a broad range of cancers. In the more common scenario, complexity rules and dictates a therapeutic strategy that relies on targeting a few crucial drivers of the oncogenic cellular phenotype. Success depends on the identification and validation of these drivers as cancer targets[176,177]. These efforts are guided by the general principle that it is easier to correct a gain of function than to restore a loss of function. Oncogenes remain very much in the line of fire.

Peter K. Vogt is at The Scripps Research Institute, La Jolla, California 92037, USA.
e-mail: pkvogt@scripps.edu

doi:10.1038/nrc3320
Published online 17 August 2012

1. Temin, H. M. & Rubin, H. Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology* **6**, 669–688 (1958).
2. Hanafusa, H., Hanafusa, T. & Rubin, H. Analysis of the defectiveness of Rous sarcoma virus. I. Characterization of the helper virus. *Virology* **22**, 591–601 (1964).
3. Hanafusa, H., Hanafusa, T. & Rubin, H. Analysis of the defectiveness of Rous sarcoma virus, II. Specification of RSV antigenicity by helper virus. *Proc. Natl Acad. Sci. USA* **51**, 41–48 (1964).
4. Rubin, H. & Vogt, P. K. An avian leukosis virus associated with stocks of Rous sarcoma virus. *Virology* **17**, 184–194 (1962).
5. Martin, G. S. The road to Src. *Oncogene* **23**, 7910–7917 (2004).
6. Temin, H. M. The control of cellular morphology in embryonic cells infected with rous sarcoma virus *in vitro*. *Virology* **10**, 182–197 (1960).
7. Yoshii, S. & Vogt, P. K. A mutant of rous sarcoma virus (type O) causing fusiform cell transformation. *Proc. Soc. Exp. Biol. Med.* **135**, 297–301 (1970).
8. Hanafusa, H., Hanafusa, T. & Rubin, H. The defectiveness of Rous sarcoma virus. *Proc. Natl Acad. Sci. USA* **49**, 572–580 (1963).
9. Temin, H. M. Separation of morphological conversion and virus production in Rous sarcoma virus infection. *Cold Spring Harb. Symp. Quant. Biol.* **27**, 407–414 (1962).
10. Goldé, A. & Lacassagne, M. Non-défectivité de la souche de virus de Rouse de Schmidt-Ruppin. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D.* **262**, 329–331 (1966) (in French).
11. Duff, R. G. & Vogt, P. K. Characteristics of two new avian tumor virus subgroups. *Virology* **39**, 18–30 (1969).
12. Martin, G. S. & Duesberg, P. H. The a subunit in the RNA of transforming avian tumor viruses. I. Occurrence in different virus strains. II. Spontaneous loss resulting in nontransforming variants. *Virology* **47**, 494–497 (1972).
13. Vogt, P. K. Spontaneous segregation of nontransforming viruses from cloned sarcoma viruses. *Virology* **46**, 939–946 (1971).
14. Martin, G. S. Rous sarcoma virus: a function required for the maintenance of the transformed state. *Nature* **227**, 1021–1023 (1970).
15. Toyoshima, K. & Vogt, P. K. Temperature sensitive mutants of an avian sarcoma virus. *Virology* **39**, 930–931 (1969).
16. Duesberg, P. H. & Vogt, P. K. Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses. *Proc. Natl Acad. Sci. USA* **67**, 1673–1680 (1970).
17. Bernstein, A., MacCormick, R. & Martin, G. S. Transformation-defective mutants of avian sarcoma viruses: the genetic relationship between conditional and nonconditional mutants. *Virology* **70**, 206–209 (1976).
18. Wang, L. H., Duesberg, P. H., Kawai, S. & Hanafusa, H. Location of envelope-specific and sarcoma-specific oligonucleotides on RNA of Schmidt-Ruppin Rous sarcoma virus. *Proc. Natl Acad. Sci. USA* **73**, 447–451 (1976).
19. Baltimore, D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* **226**, 1209–1211 (1970).
20. Temin, H. M. & Mizutani, S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* **226**, 1211–1213 (1970).
21. Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260**, 170–173 (1976).
22. Roussel, M. *et al.* Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. *Nature* **281**, 452–455 (1979).
23. Coffin, J. Hughes, S. & Varmus, H. (eds) *Retroviruses* (Cold Spring Harbor Laboratory Press, 1997).
24. Strauss, J. & Strauss, E. *Viruses and Human Disease* (Academic Press, 2007).
25. Flint, S., Enquist, L. & Racaniello, V. *Principles of Virology* (ASM Press, 2009).
26. Temin, H. Nature of the provirus of Rous sarcoma. *Natl Cancer Inst. Monogr.* **17**, 557–570 (1964).
27. Temin, H. M. The participation of DNA in Rous sarcoma virus production. *Virology* **23**, 486–494 (1964).
28. Vogt, P. & Bader, A. in *Encyclopedia of Virology* (eds Mahy, B. W. J. & van Regenmortel, M. H. V.) 445–450 (Elsevier, 2008).
29. Varmus, H. E. Form and function of retroviral proviruses. *Science* **216**, 812–820 (1982).
30. Brugge, J. S. & Erikson, R. L. Identification of a transformation-specific antigen induced by an avian sarcoma virus. *Nature* **269**, 346–348 (1977).
31. Collett, M. S. & Erikson, R. L. Protein kinase activity associated with the avian sarcoma virus src gene product. *Proc. Natl Acad. Sci. USA* **75**, 2021–2024 (1978).
32. Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E. & Bishop, J. M. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. *Cell* **15**, 561–572 (1978).
33. Hunter, T. & Sefton, B. M. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc. Natl Acad. Sci. USA* **77**, 1311–1315 (1980).
34. Ushiro, H. & Cohen, S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. *J. Biol. Chem.* **255**, 8363–8365 (1980).
35. Hunter, T. Tyrosine phosphorylation: thirty years and counting. *Curr. Opin. Cell Biol.* **21**, 140–146 (2009).
36. Czernilofsky, A. P. *et al.* Corrections to the nucleotide sequence of the src gene of Rous sarcoma virus. *Nature* **301**, 736–738 (1983).

37. Czernilofsky, A. P. *et al.* Nucleotide sequence of an avian sarcoma virus oncogene (src) and proposed amino acid sequence for gene product. *Nature* **287**, 198–203 (1980).
38. Schwartz, D. E., Tizard, R. & Gilbert, W. Nucleotide sequence of Rous sarcoma virus. *Cell* **32**, 853–869 (1983).
39. Takeya, T., Feldman, R. A. & Hanafusa, H. DNA sequence of the viral and cellular src gene of chickens. 1. Complete nucleotide sequence of an EcoRI fragment of recovered avian sarcoma virus which codes for gp37 and pp60src. *J. Virol.* **44**, 1–11 (1982).
40. Coussens, P. M., Cooper, J. A., Hunter, T. & Shalloway, D. Restriction of the *in vitro* and *in vivo* tyrosine protein kinase activities of pp60c-src relative to pp60v-src. *Mol. Cell. Biol.* **5**, 2753–2763 (1985).
41. Iba, H., Cross, F. R., Garber, E. A. & Hanafusa, H. Low level of cellular protein phosphorylation by nontransforming overproduced p60c-src. *Mol. Cell. Biol.* **5**, 1058–1066 (1985).
42. Iba, H., Takeya, T., Cross, F. R., Hanafusa, T. & Hanafusa, H. Rous sarcoma virus variants that carry the cellular src gene instead of the viral src gene cannot transform chicken embryo fibroblasts. *Proc. Natl Acad. Sci. USA* **81**, 4424–4428 (1984).
43. Sadowski, I., Stone, J. C. & Pawson, T. A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus P130gag-fps. *Mol. Cell. Biol.* **6**, 4396–4408 (1986).
44. Pawson, T. Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* **116**, 191–203 (2004).
45. Sicheri, F., Moarefi, I. & Kuriyan, J. Crystal structure of the Src family tyrosine kinase Hck. *Nature* **385**, 602–609 (1997).
46. Xu, W., Doshi, A., Lei, M., Eck, M. J. & Harrison, S. C. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. *Mol. Cell* **3**, 629–638 (1999).
47. Martin, G. S. The hunting of the Src. *Nature Rev. Mol. Cell Biol.* **2**, 467–475 (2001).
48. Duesberg, P. H., Bister, K. & Vogt, P. K. The RNA of avian acute leukemia virus MC29. *Proc. Natl Acad. Sci. USA* **74**, 4320–4324 (1977).
49. Bister, K., Ramsay, G., Hayman, M. J. & Duesberg, P. H. OK10, an avian acute leukemia virus of the MC 29 subgroup with a unique genetic structure. *Proc. Natl Acad. Sci. USA* **77**, 7142–7146 (1980).
50. Duesberg, P. H. & Vogt, P. K. Avian acute leukemia viruses MC29 and MH2 share specific RNA sequences: evidence for a second class of transforming genes. *Proc. Natl Acad. Sci. USA* **76**, 1633–1637 (1979).
51. Kan, N. C., Flordellis, C. S., Garon, C. F., Duesberg, P. H. & Papas, T. S. Avian carcinoma virus MH2 contains a transformation-specific sequence, mht, and shares the myc sequence with MC29, CMII, and OK10 viruses. *Proc. Natl Acad. Sci. USA* **80**, 6566–6570 (1983).
52. Bister, K., Hayman, M. J. & Vogt, P. K. Defectiveness of avian myelocytomatosis virus MC29: isolation of long-term nonproducer cultures and analysis of virus-specific polypeptide synthesis. *Virology* **82**, 431–448 (1977).
53. Maxam, A. M. & Gilbert, W. A new method for sequencing DNA. *Proc. Natl Acad. Sci. USA* **74**, 560–564 (1977).
54. Sanger, F. & Coulson, A. R. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* **94**, 441–448 (1975).
55. Dalla-Favera, R. *et al.* Cloning and characterization of different human sequences related to the onc gene (v-myc) of avian myelocytomatosis virus (MC29). *Proc. Natl Acad. Sci. USA* **79**, 6497–6501 (1982).
56. Alitalo, K. *et al.* Nucleotide sequence to the v-myc oncogene of avian retrovirus MC29. *Proc. Natl Acad. Sci. USA* **80**, 100–104 (1983).
57. Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. Nuclear location of the putative transforming protein of avian myelocytomatosis virus. *Cell* **29**, 427–439 (1982).
58. Blackwood, E. M. & Eisenman, R. N. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* **251**, 1211–1217 (1991).
59. Eisenman, R. N. The Max network: coordinated transcriptional regulation of cell growth and proliferation. *Harvey Lect.* **96**, 1–32 (2000).
60. Seitz, V. *et al.* Deep sequencing of MYC DNA-binding sites in Burkitt lymphoma. *PLoS ONE* **6**, e26837 (2011).
61. Ikegaki, N., Minna, J. & Kennett, R. H. The human L-myc gene is expressed as two forms of protein in small cell lung carcinoma cell lines: detection by monoclonal antibodies specific to two myc homology box sequences. *EMBO J.* **8**, 1793–1799 (1989).
62. Schwab, M. *et al.* Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* **305**, 245–248 (1983).
63. Depinho, R. A. *et al.* Myc family genes: a dispersed multi-gene family. *Ann. Clin. Res.* **18**, 284–289 (1986).
64. Dalla-Favera, R. *et al.* Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7824–7827 (1982).
65. Schwab, M. *et al.* Enhanced expression of the human gene N-myc consequent to amplification of DNA may contribute to malignant progression of neuroblastoma. *Proc. Natl Acad. Sci. USA* **81**, 4940–4944 (1984).
66. Liu, P. *et al.* Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. *Nature Med.* **17**, 1116–1120 (2011).
67. Soucek, L. *et al.* Modelling Myc inhibition as a cancer therapy. *Nature* **455**, 679–683 (2008).
68. Harvey, J. J. An unidentified virus which causes the rapid production of tumours in mice. *Nature* **204**, 1104–1105 (1964).
69. Kirsten, W. H. & Mayer, L. A. Morphologic responses to a murine erythroblastosis virus. *J. Natl Cancer Inst.* **39**, 311–335 (1967).
70. Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus. *Virology* **96**, 64–79 (1979).
71. Parks, W. P. & Scolnick, E. M. In vitro translation of Harvey murine sarcoma virus RNA. *J. Virol.* **22**, 711–719 (1977).
72. Hager, G. L. *et al.* Molecular cloning of the Harvey sarcoma virus closed circular DNA intermediates: initial structural and biological characterization. *J. Virol.* **31**, 795–809 (1979).
73. Tsuchida, N., Ryder, T. & Ohtsubo, E. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. *Science* **217**, 937–939 (1982).
74. Tsuchida, N. & Uesugi, S. Structure and functions of the Kirsten murine sarcoma virus genome: molecular cloning of biologically active Kirsten murine sarcoma virus DNA. *J. Virol.* **38**, 720–727 (1981).
75. Gibbs, J. B., Sigal, I. S., Poe, M. & Scolnick, E. M. Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules. *Proc. Natl Acad. Sci. USA* **81**, 5704–5708 (1984).
76. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. Comparative biochemical properties of normal and activated human ras p21 protein. *Nature* **310**, 644–649 (1984).
77. Scolnick, E. M., Papageorge, A. G. & Shih, T. Y. Guanine nucleotide-binding activity as an assay for src protein of rat-derived murine sarcoma viruses. *Proc. Natl Acad. Sci. USA* **76**, 5355–5359 (1979).
78. Sweet, R. W. *et al.* The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity. *Nature* **311**, 273–275 (1984).
79. Kamata, T. & Feramisco, J. R. Epidermal growth factor stimulates guanine nucleotide binding activity and phosphorylation of ras oncogene proteins. *Nature* **310**, 147–150 (1984).
80. Downward, J., Riehl, R., Wu, L. & Weinberg, R. A. Identification of a nucleotide exchange-promoting activity for p21ras. *Proc. Natl Acad. Sci. USA* **87**, 5998–6002 (1990).
81. Wolfman, A. & Macara, I. G. A cytosolic protein catalyzes the release of GDP from p21ras. *Science* **248**, 67–69 (1990).
82. McCormick, F. Signal transduction. How receptors turn Ras on. *Nature* **363**, 15–16 (1993).
83. Kan, N. C., Flordellis, C. S., Mark, G. E., Duesberg, P. H. & Papas, T. S. A common onc gene sequence transduced by avian carcinoma virus MH2 and by murine sarcoma virus 3611. *Science* **223**, 813–816 (1984).
84. Rapp, U. R. & Todaro, G. J. Generation of oncogenic mouse type C viruses: *in vitro* selection of carcinoma-inducing variants. *Proc. Natl Acad. Sci. USA* **77**, 624–628 (1980).
85. Jansen, H. W. *et al.* Homologous cell-derived oncogenes in avian carcinoma virus MH2 and murine sarcoma virus 3611. *Nature* **307**, 281–284 (1984).
86. Jansen, H. W., Ruckert, B., Lurz, R. & Bister, K. Two unrelated cell-derived sequences in the genome of avian leukemia and carcinoma inducing retrovirus MH2. *EMBO J.* **2**, 1969–1975 (1983).
87. Moodie, S. A., Willumsen, B. M., Weber, M. J. & Wolfman, A. Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. *Science* **260**, 1658–1661 (1993).
88. Vojtek, A. B., Hollenberg, S. M. & Cooper, J. A. Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell* **74**, 205–214 (1993).
89. Warne, P. H., Viciana, P. R. & Downward, J. Direct interaction of Ras and the amino-terminal region of Raf-1 *in vitro*. *Nature* **364**, 352–355 (1993).
90. Rodriguez-Viciana, P. *et al.* Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* **370**, 527–532 (1994).
91. Adari, H., Lowy, D. R., Willumsen, B. M., Der, C. J. & McCormick, F. Guanosine triphosphatase activating protein (GAP) interacts with the p21 ras effector binding domain. *Science* **240**, 518–521 (1988).
92. Scheffzek, K. *et al.* The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science* **277**, 333–338 (1997).
93. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* **300**, 149–152 (1982).
94. Tabin, C. J. *et al.* Mechanism of activation of a human oncogene. *Nature* **300**, 143–149 (1982).
95. Taparowsky, E. *et al.* Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature* **300**, 762–765 (1982).
96. Trahey, M. & McCormick, F. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. *Science* **238**, 542–545 (1987).
97. Willingham, M. C., Pastan, I., Shih, T. Y. & Scolnick, E. M. Localization of the src gene product of the Harvey strain of MSV to plasma membrane of transformed cells by electron microscopic immunocytochemistry. *Cell* **19**, 1005–1014 (1980).
98. Finegold, A. A., Schafer, W. R., Rine, J., Whiteway, M. & Tamanoi, F. Common modifications of trimeric G proteins and ras protein: involvement of polyisoprenylation. *Science* **249**, 165–169 (1990).
99. Schafer, W. R. *et al.* Genetic and pharmacological suppression of oncogenic mutations in ras genes of yeast and humans. *Science* **245**, 379–385 (1989).
100. Perucho, M. *et al.* Human-tumor-derived cell lines contain common and different transforming genes. *Cell* **27**, 467–476 (1981).
101. Shih, C., Padhy, L. C., Murray, M. & Weinberg, R. A. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. *Nature* **290**, 261–264 (1981).
102. Der, C. J., Krontiris, T. G. & Cooper, G. M. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc. Natl Acad. Sci. USA* **79**, 3637–3640 (1982).
103. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. *Nature* **297**, 474–478 (1982).
104. Santos, E. *et al.* Malignant activation of a K-ras oncogene in lung carcinoma but not in normal tissue of the same patient. *Science* **223**, 661–664 (1984).
105. Malumbres, M. & Barbacid, M. RAS oncogenes: the first 30 years. *Nature Rev. Cancer* **3**, 459–465 (2003).
106. Hall, A., Marshall, C. J., Spurr, N. K. & Weiss, R. A. Identification of transforming gene in two human sarcoma cell lines as a new member of the ras gene family located on chromosome 1. *Nature* **303**, 396–400 (1983).
107. Engelbreth-Holm, J. & Rothe Meyer, A. On the connection between erythroblastosis (Hæmocytoblastosis), myelosis and sarcoma in chicken. *APMIS* **12**, 352–365 (1935).
108. Bister, K. & Jansen, H. W. Oncogenes in retroviruses and cells: biochemistry and molecular genetics. *Adv. Cancer Res.* **47**, 99–188 (1986).
109. Bister, K. & Duesberg, P. H. Structure and specific sequences of avian erythroblastosis virus RNA: evidence for multiple classes of transforming genes among avian tumor viruses. *Proc. Natl Acad. Sci. USA* **76**, 5023–5027 (1979).
110. Lai, M. M., Hu, S. S. & Vogt, P. K. Avian erythroblastosis virus: transformation-specific sequences form a contiguous segment of 3.25 kb located in the middle of the 6-kb genome. *Virology* **97**, 366–377 (1979).

111. Hihara, H., Yamamoto, H., Shimohira, H., Arai, K. & Shimizu, T. Avian erythroblastosis virus isolated from chick erythroblastosis induced by lymphatic leukemia virus subgroup A. *J. Natl Cancer Inst.* **70**, 891–897 (1983).
112. Boerner, J. L., Danielsen, A. & Maihle, N. J. Ligand-independent oncogenic signaling by the epidermal growth factor receptor: v-ErbB as a paradigm. *Exp. Cell Res.* **284**, 111–121 (2003).
113. Vennstrom, B., Fanshier, L., Moscovici, C. & Bishop, J. M. Molecular cloning of the avian erythroblastosis virus genome and recovery of oncogenic virus by transfection of chicken cells. *J. Virol.* **36**, 575–585 (1980).
114. Yamamoto, T., Hihara, H., Nishida, T., Kawai, S. & Toyoshima, K. A new avian erythroblastosis virus, AEV-H, carries *erbB* gene responsible for the induction of both erythroblastosis and sarcomas. *Cell* **34**, 225–232 (1983).
115. Yamamoto, T. *et al.* The *erbB* gene of avian erythroblastosis virus is a member of the src gene family. *Cell* **35**, 71–78 (1983).
116. Nishida, T. *et al.* Comparison of genome structures among three different strains of avian erythroblastosis virus. *Gann* **75**, 325–333 (1984).
117. Hayman, M. J., Royer-Pokora, B. & Graf, T. Defectiveness of avian erythroblastosis virus: synthesis of a 75K gag-related protein. *Virology* **92**, 31–45 (1979).
118. Rettenmier, C. W. Anderson, S.M., Riemen, M.W. & Hanafusa, H. gag-Related polypeptides encoded by replication-defective avian oncoviruses. *J. Virol.* **32**, 749–761 (1979).
119. Hayman, M. J. *et al.* Identification and characterization of the avian erythroblastosis virus erbB gene product as a membrane glycoprotein. *Cell* **32**, 579–588 (1983).
120. Privalsky, M. L., Sealy, L., Bishop, J. M., McGrath, J. P. & Levinson, A. D. The product of the avian erythroblastosis virus *erbB* locus is a glycoprotein. *Cell* **32**, 1257–1267 (1983).
121. Hayman, M. J. & Beug, H. Identification of a form of the avian erythroblastosis virus *erb-B* gene product at the cell surface. *Nature* **309**, 460–462 (1984).
122. Privalsky, M. L. & Bishop, J. M. Subcellular localization of the v-erb-B protein, the product of a transforming gene of avian erythroblastosis virus. *Virology* **135**, 356–368 (1984).
123. Privalsky, M. L., Ralston, R. & Bishop, J. M. The membrane glycoprotein encoded by the retroviral oncogene v-erb-B is structurally related to tyrosine-specific protein kinases. *Proc. Natl Acad. Sci. USA* **81**, 704–707 (1984).
124. Kris, R. M. *et al.* Antibodies against a synthetic peptide as a probe for the kinase activity of the avian EGF receptor and v-erbB protein. *Cell* **40**, 619–625 (1985).
125. Decker, S. J. Phosphorylation of the *erbB* gene product from an avian erythroblastosis virus-transformed chick fibroblast cell line. *J. Biol. Chem.* **260**, 2003–2006 (1985).
126. Downward, J. *et al.* Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. *Nature* **307**, 521–527 (1984).
127. Ullrich, A. *et al.* Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* **309**, 418–425 (1984).
128. Hatanpaa, K. J., Burma, S., Zhao, D. & Habib, A. A. Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. *Neoplasia* **12**, 675–684 (2010).
129. Mellinghoff, I. K. *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N. Engl. J. Med.* **353**, 2012–2024 (2005).
130. Lynch, T. J. *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
131. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
132. Pao, W. *et al.* EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl Acad. Sci. USA* **101**, 13306–13311 (2004).
133. Yamamoto, H., Toyooka, S. & Mitsudomi, T. Impact of EGFR mutation analysis in non-small cell lung cancer. *Lung Cancer* **63**, 315–321 (2009).
134. Plowman, G. D. *et al.* Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc. Natl Acad. Sci. USA* **90**, 1746–1750 (1993).
135. Plowman, G. D. *et al.* Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. *Proc. Natl Acad. Sci. USA* **87**, 4905–4909 (1990).
136. Schechter, A. L. *et al.* The neu gene: an *erbB*-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science* **229**, 976–978 (1985).
137. Bargmann, C. I., Hung, M. C. & Weinberg, R. A. Multiple independent activations of the neu oncogene by a point mutation altering the transmembrane domain of p185. *Cell* **45**, 649–657 (1986).
138. Slamon, D. J. *et al.* Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235**, 177–182 (1987).
139. Cobleigh, M. A. *et al.* Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* **17**, 2639–2648 (1999).
140. Amin, D. N., Campbell, M. R. & Moasser, M. M. The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics. *Semin. Cell Dev. Biol.* **21**, 944–950 (2010).
141. Muraoka-Cook, R. S., Feng, S. M., Strunk, K. E. & Earp, H. S. ErbB4/HER4: role in mammary gland development, differentiation and growth inhibition. *J. Mammary Gland Biol. Neoplasia* **13**, 235–246 (2008).
142. Kung, H. J., Boerkoel, C. & Carter, T. H. Retroviral mutagenesis of cellular oncogenes: a review with insights into the mechanisms of insertional activation. *Curr. Top. Microbiol. Immunol.* **171**, 1–25 (1991).
143. Maki, Y., Bos, T. J., Davis, C., Starbuck, M. & Vogt, P. K. Avian sarcoma virus 17 carries the jun oncogene. *Proc. Natl Acad. Sci. USA* **84**, 2848–2852 (1987).
144. Li, J. & Vogt, P. K. The retroviral oncogene qin belongs to the transcription factor family that includes the homeotic gene fork head. *Proc. Natl Acad. Sci. USA* **90**, 4490–4494 (1993).
145. Chang, H. W. *et al.* Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. *Science* **276**, 1848–1850 (1997).
146. Curran, T. & Teich, N. M. Candidate product of the FBJ murine osteosarcoma virus oncogene: characterization of a 55,000-dalton phosphoprotein. *J. Virol.* **42**, 114–122 (1982).
147. Vogt, P. K. The story of Jun. *Arch. Biochem. Biophys.* **316**, 1–4 (1995).
148. Vogt, P. K. Jun, the oncoprotein. *Oncogene* **20**, 2365–2377 (2001).
149. Li, Z., Tuteja, G., Schug, J. & Kaestner, K. H. Foxa1 and Foxa2 are essential for sexual dimorphism in liver cancer. *Cell* **148**, 72–83 (2012).
150. Myatt, S. S. & Lam, E. W. The emerging roles of forkhead box (Fox) proteins in cancer. *Nature Rev. Cancer* **7**, 847–859 (2007).
151. Schaffhausen, B. S. & Roberts, T. M. Lessons from polyoma middle T antigen on signaling and transformation: A DNA tumor virus contribution to the war on cancer. *Virology* **384**, 304–316 (2009).
152. Whitman, M., Kaplan, D. R., Schaffhausen, B., Cantley, L. & Roberts, T. M. Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* **315**, 239–242 (1985).
153. Cantley, L. C. *et al.* Oncogenes and phosphatidylinositol turnover. *Ann. NY Acad. Sci.* **488**, 481–490 (1986).
154. Kaplan, D. R. *et al.* Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* **50**, 1021–1029 (1987).
155. Courtneidge, S. A. & Heber, A. An 81 kd protein complexed with middle T antigen and pp60c-src: a possible phosphatidylinositol kinase. *Cell* **50**, 1031–1037 (1987).
156. Whitman, M., Downes, C. P., Keeler, M., Keller, T. & Cantley, L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* **332**, 644–646 (1988).
157. Yuan, T. L. & Cantley, L. C. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* **27**, 5497–5510 (2008).
158. Engelman, J. A., Luo, J. & Cantley, L. C. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nature Rev. Genet.* **7**, 606–619 (2006).
159. Aoki, M. *et al.* The catalytic subunit of phosphoinositide 3-kinase: requirements for oncogenicity. *J. Biol. Chem.* **275**, 6267–6275 (2000).
160. Sun, M., Hart, J. R., Hillmann, P., Gymnopoulos, M. & Vogt, P. K. Addition of N-terminal peptide sequences activates the oncogenic and signaling potentials of the catalytic subunit p110α of phosphoinositide-3-kinase. *Cell Cycle* **10**, 3731–3739 (2011).
161. Chaussade, C., Cho, K., Mawson, C., Rewcastle, G. W. & Shepherd, P. R. Functional differences between two classes of oncogenic mutation in the PIK3CA gene. *Biochem. Biophys. Res. Commun.* **381**, 577–581 (2009).
162. Zhao, L. & Vogt, P. K. Helical domain and kinase domain mutations in p110α of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc. Natl Acad. Sci. USA* **105**, 2652–2657 (2008).
163. Miled, N. *et al.* Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science* **317**, 239–242 (2007).
164. Shekar, S. C. *et al.* Mechanism of constitutive phosphoinositide 3-kinase activation by oncogenic mutants of the p85 regulatory subunit. *J. Biol. Chem.* **280**, 27850–27855 (2005).
165. Georgescu, M. M. PTEN tumor suppressor network in PI3K-Akt pathway control. *Genes Cancer* **1**, 1170–1177 (2010).
166. Courtney, K. D., Corcoran, R. B. & Engelman, J. A. The PI3K pathway as drug target in human cancer. *J. Clin. Oncol.* **28**, 1075–1083 (2010).
167. Hennessy, B. T., Smith, D. L., Ram, P. T., Lu, Y. & Mills, G. B. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nature Rev. Drug Discov.* **4**, 988–1004 (2005).
168. Samuels, Y. *et al.* High frequency of mutations of the *PIK3CA* gene in human cancers. *Science* **304**, 554 (2004).
169. Maehama, T. & Dixon, J. E. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **273**, 13375–13378 (1998).
170. Li, J. *et al.* PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275**, 1943–1947 (1997).
171. Feldman, M. E. & Shokat, K. M. New inhibitors of the PI3K-Akt-mTOR pathway: insights into mTOR signaling from a new generation of Tor Kinase Domain Inhibitors (TORKinibs). *Curr. Top. Microbiol. Immunol.* **347**, 241–262 (2010).
172. Sherr, C. J. Principles of tumor suppression. *Cell* **116**, 235–246 (2004).
173. Lovat, F., Valeri, N. & Croce, C. M. MicroRNAs in the pathogenesis of cancer. *Semin. Oncol.* **38**, 724–733 (2011).
174. Morris, K. V. & Vogt, P. K. Long antisense non-coding RNAs and their role in transcription and oncogenesis. *Cell Cycle* **9**, 2544–2547 (2010).
175. Druker, B. J. Translation of the Philadelphia chromosome into therapy for CML. *Blood* **112**, 4808–4817 (2008).
176. Corcoran, R. B., Settleman, J. & Engelman, J. A. Potential therapeutic strategies to overcome acquired resistance to BRAF or MEK inhibitors in BRAF mutant cancers. *Oncotarget* **2**, 336–346 (2011).
177. Engelman, J. A. & Janne, P. A. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin. Cancer Res.* **14**, 2895–2899 (2008).
178. Hayward, W. S., Neel, B. G. & Astrin, S. M. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. *Nature* **290**, 475–480 (1981).
179. Kool, J. & Berns, A. High-throughput insertional mutagenesis screens in mice to identify oncogenic networks. *Nature Rev. Cancer* **9**, 389–399 (2009).
180. Berk, A. J. Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* **24**, 7673–7685 (2005).
181. Munger, K. *et al.* Mechanisms of human papillomavirus-induced oncogenesis. *J. Virol.* **78**, 11451–11460 (2004).
182. Bracken, A. P., Ciro, M., Cocito, A. & Helin, K. E2F target genes: unraveling the biology. *Trends Biochem. Sci.* **29**, 409–417 (2004).
183. Sullivan, C. S. & Pipas, J. M. T antigens of simian virus 40: molecular chaperones for viral replication and tumorigenesis. *Microbiol. Mol. Biol. Rev.* **66**, 179–202 (2002).
184. Massague, J. G1 cell-cycle control and cancer. *Nature* **432**, 298–306 (2004).

185. Damania, B. Oncogenic γ-herpesviruses: comparison of viral proteins involved in tumorigenesis. *Nature Rev. Microbiol.* **2**, 656–668 (2004).
186. Hecht, J. L. & Aster, J. C. Molecular biology of Burkitt's lymphoma. *J. Clin. Oncol.* **18**, 3707–3721 (2000).
187. Smith, S. M., Anastasi, J., Cohen, K. S. & Godley, L. A. The impact of MYC expression in lymphoma biology: beyond Burkitt lymphoma. *Blood Cells Mol. Dis.* **45**, 317–323 (2010).
188. Klapproth, K. & Wirth, T. Advances in the understanding of MYC-induced lymphomagenesis. *Br. J. Haematol.* **149**, 484–497 (2010).
189. Corvi, R., Savelyeva, L. & Schwab, M. Patterns of oncogene activation in human neuroblastoma cells. *J. Neurooncol* **31**, 25–31 (1997).
190. Taylor, M. D. *et al.* Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol.* **123**, 465–472 (2012).
191. Swartling, F. J. Myc proteins in brain tumor development and maintenance. *Ups. J. Med. Sci.* **117**, 122–131 (2012).
192. Scheurlen, W. G. *et al.* Molecular analysis of childhood primitive neuroectodermal tumors defines markers associated with poor outcome. *J. Clin. Oncol.* **16**, 2478–2485 (1998).
193. Huang, P. H., Xu, A. M. & White, F. M. Oncogenic EGFR signaling networks in glioma. *Sci. Signal.* **2**, re6 (2009).
194. Zavoral, M., Minarikova, P., Zavada, F., Salek, C. & Minarik, M. Molecular biology of pancreatic cancer. *World J. Gastroenterol.* **17**, 2897–2908 (2011).
195. Morris, J. P.t., Wang, S. C. & Hebrok, M. KRAS, Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. *Nature Rev. Cancer* **10**, 683–695 (2010).
196. Perez-Mancera, P. A. & Tuveson, D. A. Physiological analysis of oncogenic K-ras. *Methods Enzymol.* **407**, 676–690 (2006).
197. Poulikakos, P. I. & Rosen, N. Mutant BRAF melanomas-dependence and resistance. *Cancer Cell* **19**, 11–15 (2011).

Acknowledgements
The author extends sincere thanks to K. Bister and A. Zembrzycki for help with this paper. K. Bister read several iterations of this article, offering insightful comments and valuable suggestions. A. Zembrzycki transformed multiple disorganized drafts into a formatted manuscript. Work of the author is supported by US National Institutes of Health grants R01CA078230, R01CA153124 and R01CA151574.

Competing interests statement
The author declares no competing financial interests.estemo

FURTHER INFORMATION
Peter K. Vogt's homepage:
http://www.scripps.edu/vogt/Vogt_Lab/Home.html
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

VIEWPOINT

The influence of race and ethnicity on the biology of cancer

Brian E. Henderson, Norman H. Lee, Victoria Seewaldt and Hongbing Shen

Abstract | It is becoming clear that some of the differences in cancer risk, incidence and survival among people of different racial and ethnic backgrounds can be attributed to biological factors. However, identifying these factors and exploiting them to help eliminate cancer disparities has proved challenging. With this in mind, we asked four scientists for their opinions on the most crucial advances, as well as the challenges and what the future holds for this important emerging area of research.

In your opinion, what have been the most crucial advances in this field over the past 5 years?

Brian E. Henderson. A few years ago, my colleagues and I published an article in this journal outlining the rationale for the search for germline genetic variants that might provide insight into the aetiology of common cancers. At the time, we hypothesized that such genetic variants might reside within candidate genes in pathways thought to be aetiologically relevant[1]. We based our approach on the known variation in cancer incidence in different racial and ethnic groups. The Multiethnic Cohort Study (MEC), based in Hawaii and California, USA, had been established to use such variations in the specific rates of cancers in different ethnic groups to attempt to disentangle the environmental from the genetic contributions to cancers[2]. Thus, in the MEC, as in the general population of the United States, the rates of prostate cancer were much higher in populations of African descent and the rates of breast cancer were highest in native Hawaiians and Japanese populations[1]. In the intervening years, we have observed additional examples of such discrepancies in cancer risk, including a higher incidence of oestrogen receptor (ER)-negative breast cancer in women of African descent[3], and a higher risk of lung cancer among moderate smokers for men of African descent and native Hawaiians[4].

In large part, the candidate gene approach was not successful in explaining the differences in cancer risk within or between racial groups. However, the rapid evolution of genotyping technology created the opportunity for 'agnostic' genome-wide association studies (GWASs) in individuals with a particular cancer and in controls. In 2006, Amundadottir and colleagues published the first evidence of a prostate cancer risk locus at chromosomal location 8q24 (REF. 5). The frequency of the risk allele was higher in a small series of African American prostate cancer cases than in their European counterparts. Using admixture scanning with more detailed fine mapping, my colleagues and I published confirmatory evidence of the original locus in 8q24 (REF. 6), and Haiman *et al.* extended this work to demonstrate that there were multiple independent risk loci within a large 3 Mb span of 8q24, many of which were most common in men of African descent[7]. We concluded that these 8q24 loci could collectively explain up to 50% of the increased risk in men of African descent. Of additional interest, subsequent work has demonstrated that independent, but occasionally overlapping, loci within the 8q24 region are also associated with other cancers, including cancers of the breast, ovary, colorectum and bladder, in multiple ethnic groups.

More recently, Haiman *et al.* have published evidence of a prostate cancer susceptibility locus at 17q21 that is more specific to an African population[8] in that the frequency of the risk allele at this locus is about 5% in African men but is quite rare (<1%) in Europeans and Asians. The authors suggested that close to 10% of the increased risk of prostate cancer in men of African descent could be attributed to this risk locus.

Several susceptibility loci for breast cancer have been discovered in women, and one of these has been specifically linked to women of African descent. A common variant at the TERT-CLPTM1L locus has been associated with ER-negative breast cancer, and the risk allele is twice as common in African American women as in their European counterparts[9].

Additional GWASs of African American, Japanese and Chinese cancer cases and controls have been published or are currently underway. Undoubtedly, additional genetic variants will be identified with allelic frequencies that vary with the cancer risk between different populations.

Norman H. Lee. The implementation of genomic approaches (for example, GWASs, gene expression and epigenomic profiling, and next-generation sequencing) over the past 5 years has reinvigorated attention into the biological component of cancer health