Urol Res (1988) 16:333–339

Urological Research

Invited Review

Oncogenes and Urological Malignancies: Implications for the Future

J. A. Schalken[1], A. M. W. van den Ouweland[2], F. M. J. Debruijne[1], H. F. M. Karthaus[3], and W. J. M. Van de Ven[2]

[1]Urological Research Laboratory, Department of Urology and [2]Molecular Oncology Section, Department of Biochemistry, University of Nijmegen, and [3]Department of Urology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

Accepted: June 1, 1988

Tumor genesis and tumor progression can be considered as an evolutionary process during which a cell gradually escapes from a delicately balanced molecular structure. In this multistep process, a number of genes are affected, amongst which are the proto-oncogenes. Under normal physiological conditions, these genes code for parts of the molecular communication network of the cells and, therefore, control cell growth and biological development. Proto-oncogenes can be turned into dominant cancer genes or oncogenes. They possess a dormant malignant capacity which can be triggered by a number of molecular mechanisms. In this paper, characteristics of oncogenes and their possible role in urological malignant disorders will be discussed.

Identification and Isolation of Oncogenes

The molecular basis of neoplasia has been a topic of intensive research during the past few decades. The relationship between DNA aberrations caused by mutagenic agents, and carcinogenesis was substantiated by developments in the investigation of tumor viruses. The study of RNA tumor viruses in particular has enhanced our knowledge on the mutability of genetic elements. These viruses have served as important tools for the study of malignant transformation. Two groups of RNA tumor viruses are known to exist; the slowly and the acutely transforming retroviruses. The latter of these as well as the DNA tumor viruses have common genetic elements, called viral oncogenes (v-*onc*), that furnish them with their oncogenic potential. The characterization of these genes and their subsequent isolation by means of recombinant DNA techniques, have been an important step in the understanding both of cellular growth and differentiation and also of the deregulation of these processes, which is probably the cause of cancer.

The present excitement about viral oncogenes and human cancer began when genetic sequences were found in the genomes of higher eukaryotes [3, 17, 60] which were homologous to retroviral *onc* genes. The recombination between a slowly transforming RNA tumor virus and its host genome [73] may result in the generation of acutely transforming retroviruses. Thus, the genesis of viral transforming capacity indicates a way to identify potentially oncogenic sequences, known as proto-oncogenes [61].

Another approach that led to the identification of cellular oncogenes was based on proviral insertion: after integration of their genome in the host genome, slowly transforming retroviruses may activate cellular genes which can lead to the deregulation of key functions in the cell and, subsequently, to the development of a tumor. In fact, this explains the slow onset of disease after infection by these viruses. Once this elusive principle was recognized [26], comparison of integration sites of mouse mammary tumorviruses led to the discovery of *int*-1 [48] and later to an entire new set of proto-oncogenes.

The assumption that oncogenes are implicated in tumorigenesis is not only based upon the characteristics of viral oncogenes. Transfection of DNA from a variety of human tumor cells resulted in malignant transformation of the recipient mouse 3T3 cells [56]. In many tumors, amplification of proto-oncogene sequences is often observed resulting in elevated levels of expression. Furthermore, in chromosomal aberrations associated with particular forms of cancer, proto-oncogenes seem to be directly involved. In the case of Burkitt's lymphoma, expression of the *myc* proto-oncogene is affected by translocated immunoglobulin enhancer sequences [50, 77]. As another example, in chronic myelocytic leukemia and acute lymphocytic leukemia cells, *abl* proto-oncogene sequences on chromosome 9 are fused to *bcr* sequences of chromosome 22 which results in the genesis of fusion proteins [23, 25]. These observations support the importance of oncogenes in the onset and development of cancer.

Function of Proto-Oncogenes

The various approaches mentioned above have revealed the existence of more than fifty proto-oncogenes. The normal

physiological and pathological role of these genes is the focus of molecular oncology research. Most proto-oncogene products characterized so far are recognisable as part of an elaborate molecular communication system.

They are linked to transduction, exertion or reception of growth signals, as is shown in the following description which follows the chain of events from an extracellular stimulus to the response of the triggered cell:

Growth Factors

An extracellular stimulus is often provided by polypeptide growth factors. A proto-oncogene that encodes a growth factor is the *sis* proto-oncogene. It encodes a protein that is very similar to the B chain of platelet-derived growth factor (PDGF) [9, 11, 69]. Growth factors play a key role in cell proliferation.

Part of our knowledge on cell growth in vivo and the signals controlling these processes has been derived from studies on established cell lines. Often these cultured cells require serum in their media for their propagation. Serum can be replaced by specific polypeptides, called growth factors [2]. Growth factors regulate cell proliferation through binding to specific cell membrane receptors [27]. They are present in a variety of tissues and are released by many cells in culture [42, 46, 51]. The fine tuning of proliferation rates necessary for coordinated growth of various cell types to form and maintain tissues might be due to the great diversification of growth factors, the receptor dependent cell type specific action of these factors and the requirement of multiple growth factors for stimulation of a specific cell [2, 22, 27, 31, 63, 68, 71].

Cell growth, however, is not necessarily under control of positive regulating forces alone. For example, exogenous tumor growth factor-β (TGF-β) inhibits the growth of many cell types including neoplastic cells. Failure to express or respond to specific growth inhibitory factors that are released by cells to regulate orderly growth may also lead to disorder. There may be various possible causes of such a failure, including mutation or loss of the structural gene for TGF-β itself, loss of positive transcriptional or translational controls for expression of TGF-β or defects in the specific cellular receptor for TGF-β. This alternative view, called "The Yin-Yang theory of cancer", inspired research on cloning tumor-suppressor genes to gain more information to support this theory [34, 41, 42, 59].

Growth Factor Receptors

As already indicated above, an important mechanism for growth factors to transmit their signal further into a cell is by binding to highly specialized receptor molecules. Studies on receptors at the structural level and their relation to proto-oncogenes have revealed intriguing phenomena that might have implications for cancer research.

The linkage between receptors and proto-oncogene products was established by the discovery that v-*erb*-B encodes a truncated version of the epidermal growth factor receptor [12, 64]. The truncation, caused by retroviral transduction, is likely to be the step that has led to the activation of this gene. Likewise, Sherr et al. [55] showed that the c-*fms* proto-oncogene encodes a membrane bound tyrosine kinase, that appeared to be closely related or identical to the colony stimulating factor (CSF-1) receptor, and is activated by a truncation at the C-terminus [8].

Detailed comparison of amino acid sequences of receptor molecules showed that most of them contain one or more characteristic domains depending on the type of receptor. Important receptor features are the kinase domain, the transmembrane domain, which is sometimes present in multiple copies, and the cysteine-rich domain, which can also be present in multiple copies. A number of receptors contain structurally similar kinase domains and cysteine-rich regions, suggesting that these arose by duplication and shuffling of the same ancestral genetic sequences. In the human insulin receptor, hIR [65], and the v-*erb*-B oncogene related human epidermal growth factor receptor, hER [64], for example, the tyrosine kinase domains and cysteine-rich regions are structurally similar. The latter ones show also a high degree of homology with the cysteine-rich domain of the low density lipoprotein receptor [62, 74], a socalled coated pit receptor.

The c-*fms* proto-oncogene product and the PDGF-receptor both lack such a cysteine-rich domain. The tyrosine kinase domain of these proteins differ from hIR and hER in that the phosphorylation acceptor site is separated by a spacer region from the catalytic domain of the enzyme. The c-*kit* proto-oncogene product has the same features [8, 75].

Some non-membrane bound hormone receptors, like the glucocorticoid receptor [28], the thyroid hormone receptor [72] and the oestrogen receptor [24] have homologous cysteine-rich repeats that are related to that of the v-*erb*-A oncogene [24, 52, 72].

Another group of membrane receptors have as characteristic feature their coupling to G-proteins. These include receptors such as the light "receptor" rhodopsin and the β_2-adrenergic receptor [10, 36], which are structurally homologous [57]. The *mas* oncogene probably belongs to this class of receptors. Its translation product has a hydrophobicity pattern that is strikingly similar to that of rhodopsin and this may reflect structural and functional similarities in these proteins [76].

Recently, a new "super-family" of receptors was molecularly characterized, among which the chemically gated ion-channel receptors, like the γ-amino butyric acid(GABA)/diazepine receptor and the nicotinic acetylcholine (nACh) receptor. They also exhibit structural homology [54]. Another class of membrane bound receptors are the integrins [30], which function as receptors for extracellular matrix proteins. A link to proto-oncogenes of these last two classes of receptors remains to be established. However, if they are involved in activating a critical component in a growth

regulatory pathway, perhaps by serving in signal transduction or as a membrane channel, they may also attain proto-oncogene status.

An issue that still remains, pertains to the mechanisms that lead to mobilization of the oncogenic potential of receptor genes which was a question addressed by recent studies. It is well known that receptors themselves are subject to various forms of regulation which influence their biological activity, conformation and subcellular distribution. Interaction of the receptor with its ligand, for instance, may induce conformational changes which then may trigger a profilerative response. In a number of plasma membrane receptors, it was shown that phosphorylation plays a key role in this. It is, therefore, conceivable that alterations in receptor structure and/or changes in phosphorylation events lead to escape from regulation of normal receptor function and, because of that, to triggering of oncogenic behavior. As an example, the epidermal growth factor receptor is briefly discussed. As mentioned before, EGF-receptor truncation apparently contributes to mobilization of oncogenic potential. This was concluded from comparative studies of the viral oncogene v-*erb*-B, which encodes an EGF-receptor-related protein that misses the major part of the extracellular ligand-binding domain, and the EGF-receptor itself. It was suggested that the primary physiological function of EGF was induction of conformational changes in EGF-receptor molecules, possibly by cross-linking them. Following such an activation step, all the requirements necessary for triggering a proliferative response in a cell reside in the receptor itself. Involved in this response is probably another intrinsic function of the receptor, namely its ability to phosphorylate tyrosine residues. This activity is controlled by various regulatory mechanisms. The activity can be enhanced, for instance, by autophosphorylation (e.g. upon ligand binding) via an intramolecular mechanism and can be suppressed by protein kinase C. In the case of the v-*erb*-B protein, normal physiological activation by growth factor binding is impossible because the EGF-binding domain is absent. Nevertheless, the protein apparently possesses all the requirements for triggering a proliferative response with oncogenic features. It is possible that the different structure of the v-*erb*-B protein, induced by truncation of the cellular EGF-receptor, mimics the EGF activation step, implicating the conformational change as a crucial event in mobilizing the oncogenic potential of the EGF-receptor [12, 21].

A more complete picture of the link between certain oncogenes and genes that encode receptors is emerging. At the moment most receptor genes are essentially amenable to activation mechanisms, comparable to the one described above. For instance, it was shown that the insulin receptor function could be influenced by substition of the internal twin tyrosine residues that are involved in receptor phosphorylation [14]. Therefore, it is possible that many receptor encoding genes are candidate proto-oncogenes, considered "innocent only until found guilty".

GTP-Binding Protein

In the process of signal transduction into the cell, the *ras* proto-oncogene family is probably involved at the cell membrane level. Both in structure and in GTP-binding characteristics, they resemble G-proteins [18, 38]. Their precise role in the signal transduction pathway is not yet known (for review, see [1]).

Protein Kinases

In the cytoplasm, the products of proto-oncogenes that encode protein kinases are elements in the signal transduction pathway. By far the largest group of oncogenes with a common functional domain, is that of the tyrosine kinase encoding genes. Whereas some of their gene products are membrane bound and have a receptor function (see previous section), the function of others is more obscure. They have no structural characteristics that would allow membrane attachment and are therefore called cytoplasmic tyrosine kinases (for a review, see [29]). Nevertheless, sometimes they appeared to be localized at the plasmamembrane. In the case of $pp60^{c\text{-}src}$, myristilation at the N-terminus might be instrumental in membrane attachment [6, 32]. The way in which the v-*fes* product is attached to the membrane [15, 16, 43] has not been elucidated. The *gag*-p15 portion might be responsible for this phenomenon [66]. Since phosphorylation of receptors is an important step in the regulation of the function of these molecules [57], tyrosine kinases are also likely to be implicated in the signal transduction pathway. Aberrations in the functioning of these molecules is presumably the step that eventually could lead to transformation.

Proto-Oncogene Products in Cell Nucleus

The products of some proto-oncogenes are found in the nucleus. These include the products of the proto-oncogene *fos* and *myc*. Transcription of these genes is increased by stimulation by growth factors and, in their turn, the products of *myc* and *fos* may regulate the transcription of other genes necessary for stimulation of cell proliferation [7, 35, 45].

Recently, a new viral oncogene, named *jun*, was discovered in an avian sarcoma virus isolate, designated ASV17 [5]. From immunological studies it appeared that the product of the viral oncogene was located in the nucleus of cells transfected with ASV17 DNA. Furthermore, the v-*jun* protein exhibited enhancer binding properties just like AP-1, a human transcription factor that controls growth properties of cells by governing gene regulation. The observation that the *jun* product resembles a factor that regulates gene expression (AP-1) is intriguing, especially since AP-1 can be activated by phorbol ester tumor promoters.

As stated before the malignant potential of proto-oncogenes can be mobilized only upon genetic modification which leads to qualitative and/or quantitative differences in proto-oncogene expression. It is not difficult to conceive that disturbances in the delicately balanced molecular communication network of the cell could lead to uncontrolled proliferation and pathological differentiation, poignant characteristics of cancer cells. A dilemma arises: When should a cellular gene be regarded as being a proto-oncogene? Up to now the definition is confined to those genes with an oncogenic reputation as established by retroviral transduction, enhancer or promoter insertion and transfection assays. It is likely, however, that other genes important for tumorigenesis were elusive, in that they did not present themselves as oncogenes according to the approaches outlined above.

The Role of Proto-Oncogenes in Urological Malignancies

To study proto-oncogenes, essentially three approaches are available. Firstly, the genetic structure of a proto-oncogene can be studied enabling discrimination between a normal and mutated gene. Secondly, the rate of transcription can be determined using mRNA analysis by Northern blot assays. Finally, since antibodies against oncogene proteins ("onco-proteins") have become available, the proto-oncogenes can also be studied at the protein level.

It appears, however, that most studies on oncogenes in urological malignancies, are confined to only one of the above mentioned approaches. Hence, data are mostly incomplete, with regard to the role of proto-oncogenes as etiological agent in carcinogenisis of the urogenital tract.

Oncogenes and Primary Bladder Tumors

The oncogene that might be implicated in bladder cancer is the c-Ha-*ras* 1 gene. Fuijta et al. [20] and Malone et al. [40] showed that it is activated by a point mutation, in a number of bladder tumors. The activating point mutations are around codon 12 and codon 61 and these result in a dramatic change of the properties of the p21 – *ras* – oncogene product [1]. It should be noted, however, that less time consuming methods for the detection of point mutations, as described by Bos et al. [4], are likely to reveal a greater number of activated *ras* oncogenes.

Whether *ras* p21 expression can serve as diagnostic marker for bladder cancer remains unknown.

Oncogenes in Primary Testis Tumors

The data on oncogene expression in testis tumors are also rather limited. It was shown that the p62 *myc* protein was expressed at higher levels in highly differentiated teratomas [70]. An additional aspect of this marker is that the *myc* protein is localized in the nucleus. Therefore, it is possible to use nuclei, isolated from paraffin embedded tumor specimens, for a two parameter FCM analysis by which both DNA (PI labeling) and *myc* content (anti p62 *myc* labeling) can be determined [70].

Oncogenes in Primary Prostate Cancer

Using antibodies against c-*myc*, it was shown that *myc* expression was significantly elevated in prostate cancer as compared to normal prostate tissue or benign prostatic hyperplasia [19]. Viola et al. [67] have shown that *ras* p21 protein levels increased with increasing degree of nuclear anaplasia. Moreover, they showed that there was an inverse relation between *ras* p21 expression and the degree of glandular differentiation. However, no real additional insights for the prognosis of prostatic cancer using this marker is yet provided. Another possible implication of *ras* in prostate cancer, was found by Peehl et al. [49]. They identified an activated c-Ki-*ras* oncogene by a transfection assay using DNA, isolated from a primary prostatic tumor.

Oncogene Expression in Primary Renal Cancers

In the nephroblastoma/Wilms tumor, an elevated level of N-*myc* mRNA was found [47]. For renal cell carcinoma, chromosome 3, might be implicated in tumorigenesis, since aberrant forms of it are often observed [37]. In a hereditary form of renal cell carcinoma, where a translocation between chromosomes 3 and 8 was found (t(3, 8)), the *c-myc* gene appeared to be involved in the translocation [13]. An interesting observation by Karthaus et al. [33] was the fact that in 2/15 renal cell carcinomas, the *fes* oncogene was expressed at detectable levels. Further studies are necessary, however, to establish the precise role of the expression of this oncogene in renal tumors.

In general, we can say that *myc* and *ras* oncogenes might be involved in tumorigenesis of the urogenital tract. It should be noted, however, that these two oncogenes are found to be expressed at high levels in a great number of different tumors [58]. Only limited data on oncogenes in urological malignancies are yet available, hence, the number and identity of the oncogenes that are etiologically involved in carcinogenesis of the urogenital tract remains to be established.

Another complicating factor, in the study of the molecular basis of neoplasia is the fact that the number of proto-oncogenes is likely to be greater than fifty. This makes the survey for specific genetic aberrations in carcinogenesis more complicated and a more direct approach for this problem seems to be necessary.

Implications for Future Research

For several reasons, the original enthusiasm about the role of oncogenes in malignancies has been tempered. First,

despite the overwhelming amount of circumstantial evidence that oncogenes are implicated in carcinogenesis, the precise role of most of them in tumorigenesis is still obscure. Furthermore, the number of proto-oncogenes is much larger than was appreciated. The observation that many oncogenes encode proteins involved in signal transduction and regulation of transcription, makes it tempting to believe that many more are actually oncogenes but "remain innocent until found guilty". Last but not least, results of recent studies emphasize the importance of a category of recessive genes the so-called tumor suppressor genes or anti-oncogenes. It has been suggested that this group of genes is equally important as the dominantly acting oncogenes [34]. It is conceivable that in addition to oncogenes and anti-oncogenes, also other genes are important to understand the molecular basis of neoplasia. Additional approaches are likely required to identify such genes.

A useful method to isolate and characterize genetic elements that are functionally related to a specific malignancy and that might as well be useful as marker, is to differentiate at the level of gene expression by differential hybridization analysis of cDNA libraries; cDNA libraries are constructed of mRNA isolated from cells at specific stages of development, growth of the carcinogenic process. Upon screening such libraries with stage-specific 32P-labeled mRNA, genes can be isolated whose expression is specific for one of these stages.

This method revealed a number of genes specifically activated during growth- and/or immunological stimulation [44]. Imperative for this approach is that the phases that are compared are developmentally similar. This can be achieved by a controlled stimulation, like EGF stimulation on NIH 3T3 cells [44], serum starvation [39] or a hydroxyurea block to synchronize cells. In the study of malignant disorders, subtraction hybridization will usually result in a large number of cDNA clones from differentially expressed genes, and therefore, a tumor model system in which specific phenotypic characteristics are represented in stable cell lines or tumor lines, is essential for such an approach.

With respect to tumors of the urogenital tract, a well described tumor model system that meets a number of criteria for differential hybridization analysis is the Dunning R-3327 rat prostatic cancer model system. It consists of more than ten tumor sublines, all derived from the original Dunning R-3327 tumor, and corresponding cell lines derived therefrom. All are characterized by parameters such as hormone dependency, histology and/or metastatic behavior. The first studies with this model system using this approach, revealed already that fibronectin is down modulated in metastasizing prostatic cancer cells [53]. Many new aspects of carcinogenesis are expected to be defined at the gene expression level in the future by this approach, that is potential useful to both characterize dominantly active genes, such as oncogenes, as well as recessive, suppressor genes, and might therefore be a very important tool in future studies on carcinogenes or the urogenital tract.

Acknowledgement. We would like to thank Mrs. M. Van Beckhoven for preparing the manuscript. This work was supported by the Dutch Cancer Foundation (Nuke-87-09 8 87-13) and the Dutch Kidney Foundation (C. 85-563 8 G85-531).

References

1. Barbacid M (1987) *ras* Genes. Ann Rev Biochem 56:779–827
2. Barnes D, Sato G (1980) Serum free cell culture: A unifying approach. Cell 22:649–655
3. Bishop JM (1983) Cellular oncogenes and retroviruses. Ann Rev Biochem 52:301–354
4. Bos JL, Toksoz D, Marshall CJ, Verlaan-deVries M, Veeneman GH, van der Eb AJ, van Boom JH, Janssen JWG, Steenvoorden ACM (1985) Amino-acid substitutions at codon 13 of the N-*ras* oncogene in human acute myeloid leukaemia. Nature 315:726–730
5. Bos TJ, Bohmann D, Truchie H, Than R, Vogt PK (1988) V-*jun* encodes a nuclear protein with en cancer binding properties of AP. 1. Cell 52:705–716
6. Buss JE, Kamps MP, Gould K, Sefton BM (1986) The absence of myristic acid decreases membrane binding of p60 *src* but does not affect tyrosine protein kinase activity. J Virol 58:468–474
7. Cochran BH, Zullo J, Verma IM, Stiles CD (1984) Expression of the c-*fos* gene and an *fos*-related gene is stimulated by platelet-derived growth factor. Science 226:1080–1082
8. Coussens L, Van Beveren C, Smith D, Chen E, Mitchell RL, Isacke CM, Verma IM, Ullrich A (1986) Structural alteration of viral homologue of receptor protooncogene *fms* at carboxyl terminus. Nature 320:277–280
9. Deuel TF, Huang JS, Huang SS, Stroobant P, Waterfield MD (1983) Expression of a platelet-derived growth factor-like protein in simian sarcoma virus transformed cells. Science 221:1348–1350
10. Dixon RAF, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Benett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ, Strader CD (1986) Cloning of the gene and cDNA for mammalian β-adrenergic receptor and homology with rhodopsin. Nature 321:75–79
11. Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades HN (1983) Simian sarcoma virus onc gene, v-*sis*, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221:275–277
12. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD (1984) Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. Nature 307:521–527
13. Drabkin HA, Bradley C, Hart I, Bleskan J, Li FP, Patterson D (1985) Translocation of c-*myc* in the heriditary renal cell carcinoma associated with a t(3; 8) (p14.2; q24.13) chromosomal translocation. Proc Natl Acad Sci USA 82:6980–6984
14. Ellis L, Clauser E, Morgan DO, Edery M, Roth RA, Rutter WJ (1986) Replacement of insulin receptor tyrosine residues 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. Cell 45:721–732
15. Feldman RA, Wang L, Hanafusa H, Balduzzi PC (1983) Avian sarcoma virus UR2 encodes a transforming protein which is associated with a unique protein kinase activity. J Virol 42:228–236
16. Feldman RA, Gabrilove JL, Tam JP, Moore MAS, Hanafusa H (1985) Specific expression of the human cellular *fps/fes*-encoded protein NCP92 in normal and leukemic myeloid cells. Proc Natl Acad Sci USA 82:2379–2383
17. Fishinger PJ (1982) In: Stephenson JR (ed) Molecular biology of RNA tumor viruses. Academic Press, New York, pp 162–250

18. Fleischman LF, Chahwala SB, Cantley L (1986) *Ras*-transformed cells: Altered levels of phosphatidylinositol-4,5-bisphosphate and catabolites. Science 231:407–410
19. Fleming WH, Hamel A, MacDonald R, Ramsey E, Pettigrew NM, Johston B, Dodd JG, Matusik RJ (1986) Expression of the c-*myc* protooncogene in human prostatic carcinoma and benign prostatic hyperplasia. Cancer Res 46:1535–1538
20. Fujita J, Srivastava SK, Kraus MH, Rhim JS, Tronick SR, Aaronson SA (1985) Frequency of molecular alterations affecting *ras* proto-oncogenes in human urinary tract tumors. Proc Natl Acad Sci USA 82:3849–3853
21. Gill GN, Bertics PJ, Thompson DM, Weber W, Cochet C (1985) In: Feramisco J, Ozanne B, Stiles C (eds) Growth factors and transformation. Cold Spring Harbor, New York, pp 11–18
22. Goustin AS, Leof EB, Shipley GD, Moses HL (1986) Growth factors and cancer. Cancer Res 46:1015–1029
23. Goyns MH (1983) Mapping of the c-*sis* oncogene on human chromosome 22 with respect to the breakpoint associated with chronic myeloid leukaemia. Biosci Rep 3:1107–1111
24. Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, Chambon P (1986) Human oestrogen receptor cDNA: Sequence, expression and homology to v-*erb*-A. Nature 320:134–139
25. Groffen J, Heisterkamp N, Stephenson JR, Geurts van Kessel A, De Klein A, Grosveld G, Bootsma D (1983) c-*sis* is translocated from chromosome 22 to chromosome 9 in chronic myelocytic leukemia. J Exp Med 158:9–15
26. Hayward WS, Neel BG, Astrin SM (1981) Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature 290:475–480
27. Heldin CH, Westermark B (1984) Growth factors: Mechanism of action and relation to oncogenes. Cell 37:9–20
28. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM (1985) Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 318:635–641
29. Hunter T (1984) The protein of oncogenes. Sci Am 251:60–69
30. Hynes RO (1987) Integrins: A family of cell surface receptors. Cell 48:549–554
31. Johnsson A, Betsholtz C, Heldin CH, Westermark B (1986) The phenotypic characteristics of simian sarcoma virustransformed human fibroblasts suggest that the v-*sis* gene product acts solely as a PDGF receptor agonist in cell transformation. EMBO J 5:1535–1541
32. Kamps MP, Buss JE, Sefton BM (1985) Mutation of NH2 terminal glycine of p60 *src* prevents both myristoylation and morphological transformation. Proc Natl Acad Sci USA 82: 4625–4628
33. Karthaus HFM, Schalken JA, Feitz WFJ, Debruyne FMJ, de Haan PT, Bloemers HPJ, Van de Ven WJM (1986) Expression of the human *fes* cellular oncogene in renal cell tumors. Urol Res 14:123–127
34. Klein G (1987) The approaching era of the tumor suppressor genes. Science 238:1539–1545
35. Kruijer W, Cooper JA, Hunter T, Verma IM (1984) Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. Nature 312:711–716
36. Kubo T, Fukuda K, Mikami A, Maeda A, Takahashi H, Mishina M, Haga T, Haga K, Ichiyama A, Kangawa K, Kojima M, Matsuo H, Hirose T, Numa S (1986) Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323:411–416
37. Kovacs G, Szucs S, De Riese W, Baumgartel H (1987) Specific chromosome aberration in human renal cell carcinoma. Int J Cancer 40:171–178
38. Lacal JC, Scrivastava SK, Anderson PS, Aaronson SA (1986) *Ras* p21 proteins with high or low GTPase activity can efficiently transform NIH/373 cells. Cell 44:609–617
39. Lau LF, Nathans D (1985) Identification of a set of genes expressed during the GO/G1 transition of cultured mouse cells. EMBO J 4:3145–3151
40. Malone PR, Visvanathan KV, Ponder BA, Shearer RJ, Summerhayes LC (1985) Oncogenes and bladder cander. Br J Urol 57:664–667
41. Marx JL (1986) The yin and yang of cell growth control. Science 232:1093–1095
42. Massagué J (1985) The transforming growth factors. TIBS 10:237–240
43. Mathey-Prevot B, Hanafusa H, Kawai S (1982) A cellular protein is immunologically crossreactive with and functionally homologous to the fujinami sarcoma virus transforming protein. Cell 28:897–906
44. Matrisian LM, Glaichenhaus N, Gesnel MC, Breathnach R (1985) Epidermal growth factor and oncogenes induce transcription of the same cellular mRNA in rat fibroblasts. EMBO J 4:1435–1440
45. Müller R, Bravo R, Burckhardt J, Curran T (1984) Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature 312:716–720
46. Nilsson J, Sjölund M, Palmberg L, Thyberg J Heldin CH (1985) Arterial smooth muscle cells in primary culture produce a platelet-derived growth factor-like protein. Proc Natl Acad Sci USA 82:4418–4422
47. Nisen PD, Zimmerman KA, Cotter SV, Gilbert F, Alt FW (1986) Enhanced expression of the N-*myc* gene in Wilms' tumors. Cancer Res 46:6217–6222
48. Nusse R, Varmus HE (1982) Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31:99–109
49. Peehl DM, Wehner N, Stamey TA (1987) Activated Ki-*ras* oncogene in human prostatic adenocarcinoma. Prostate 10: 281–289
50. Rabbitts TH, Forster A, Hamlyn P, Bear R (1984) Effect of somatic mutation within translocated c-*myc* genes in Burkitt's lymphoma. Nature 309:592–597
51. Rall LB, Scott J, Bell GI, Grawford RJ, Penschow JD, Niall HD, Coghlan JP (1985) Mouse prepo-epidermal growth factor synthesis by the kidney and other tissues. Nature 313:228–231
52. Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennstrom B (1986) The c-*erb*-A protein is a high-affinity receptor for thyroid hormone. Nature 324:635–640
53. Schalken JA, Ebeling SB, Isaacs JT, Treiger B, Bussemakers MJG, de Jong MEM, Van de Ven WJM (1988) Down modulation of fibronectin messenger RNA in metastasizing rat prostatic cancer cells revealed by differential hybridisation analysis. Cancer Res 40:2042–2046
54. Schofield PR, Darlison MG, Fujita N, Burt DR Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA (1987) Sequence and functional expression of the GABAA receptor shows a lignand-gated receptor super-family. Nature 328:221–227
55. Sherr CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanly RE (1985) The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell 41:665–676
56. Shih C, Padhey LC, Murray M, Weinberg RA (1981) Transforming genes of carcinomas and neuroblastomas introduces into mouse fibroblasts. Nature 290:261–264
57. Sibley DR, Benovic JL, Caron MG, Lefkowitz JL (1987) Regulation of transmembrane signaling by receptor phosphorylation. Cell 48:913–922
58. Slamon DJ, Cline JJ (1984) Expression of cellular oncogenes during embryonic and fetal development of the mouse. Proc Natl Acad Sci USA 81:7141
59. Sporn MB, Roberts AB (1985) Autocrine growth factors and cancer. Nature 313:745–747
60. Stehelin D, Varmus HE, Bishop JM, Vogt PK (1976) DNA

related to the transforming gene(s) of avian sarcoma virus is present in normal avian DNA. Nature 260:170–174
61. Stephenson JR, Khan AS, Van de Ven WJM, Reynolds FH (1979) Type C retroviruses as vectors for cloning cellular genes with probable transforming function. J Natl Cancer Inst 63:1111–1119
62. Sudhof TC, Goldstein JL, Brown MS, Russell DW (1985) The LDL receptor gene: a mosaic of exons shared with different proteins. Science 228:815–822
63. Tsao MC, Walthall BJ, Ham RG (1982) Clonal growth of normal human epidermal keratinocytes in a defined medium. J Cell Phys 110:219–229
64. Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Tam AW, Lee J, Yarden Y, Libermann TA, Schlessinger J, Downward J, Mayes ELV, Whittle N, Waterfield MD, Seeburg PH (1984) Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 309:418–425
65. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzelli LM, Dull TJ, Gray A, Coussens L, Liao Y-C, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761
66. Van de Ven WJM, Vermorken AJM, Onnekink C, Bloemers HPJ, Bloemendal H (1978) Structural studies on rauscher murine leukemia virus: Isolation and characterization of viral envelopes. J Virol 27:595–603
67. Viola MV, Fromowitz F, Oravez S, Deb S, Finkel G, Lundy J, Hand P, Thor A, Schlom J (1986) Expression of *ras* oncogene p21 in prostate cancer. N Engl J Med 314:133–137
68. Walthall BJ, Ham RG (1981) Multiplication of human diploid fibroblasts in a synthetic medium supplemented with EGF, insulin and dexamethasone. Exp Cell Res 134:303–311
69. Waterfield MD, Scrace GT, Whittle N, Stroobant P, Johnsson A, Wasteson A, Westermark B, Heldin CH, Huang JS, Deuel TF (1983) Platelet-derived growth factor is structurally related to the putative transforming protein p28*sis* of simian sarcoma virus. Nature 304:35–39
70. Watson JV, Stewart J, Evan GI, Ritson A, Sikora K (1986) The clinical significance of flow cytometric *c-myc* oncoprotein quantitation in testicular cancer. Br J Cancer 53:331–337
71. Weinberg RA (1985) The action of oncogenes in the cytoplasm and nucleus. Science 230:770–776
72. Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM (1986) The *c-erb*-A gene encodes a thyroid hormone receptor. Nature 324:641–646
73. Weiss R, Teich N, Varmus H, Coffin J (1986) In: RNA tumor viruses. Cold Spring Harbor, New York
74. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW (1984) The human LDL receptor: A cysteine-rich protein with multiple alu sequences in its mRNA. Cell 39:27–38
75. Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Fried VA, Ullrich A, Williams LT (1986) Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 323:226–232
76. Young D, Waitches G, Birchmeier C, Fasano O, Wigler M (1986) Isolation and characterization of a new cellular oncogene encoding a protein with multiple potential transmembrane domains. Cell 45:711–719
77. Yunis J (1983) The chromosomal basis of human neoplasia. Science 221:227:236

Dr. J. A. Schalken
Urological Research Laboratory
Department of Urology
University of Nijmegen
NL-6525 GA Nijmegen
The Netherlands