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Expression of mRNAs of Multiple Growth Factors and Receptors by Neuronal Cell Lines: Detection with RT-PCR

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Neurons and glia are capable of both secreting and responding to a large variety of growth factors. However, information on multiple expression of growth factors and their receptors was usually obtained from uncorrelated observations, using cells from various animals of origin, developmental stages, growth phases, culture ages and culture conditions. Because of its specificity and extreme sensitivity, reverse transcription-polymerase chain reaction (RT-PCR) is uniquely suitable to study a large panel of growth factors and their receptors from a limited cell sample, free of these intervening variables. In this paper we evaluate the expression of mRNA of a total of 35 growth factor-related proteins by conducting RT-PCR on three neuronal cell lines: the PC12 rat pheochromocytoma line, the MAH rat sympathoadrenal progenitor line, and the N18 mouse neuroblastoma line. Three types of results are presented. The first confirms the existing knowledge such as the presence of Trk-A (NFG receptor) in PC12. The second consists of new information that expands and extends earlier observations, such as the presence of CNTF receptor complex in PC12, which explains our previous report that CNTF enhances the biological effects of NGF on these cells. The third consists of novel information that leads the way to further experimentation by the more conventional methods. These include the strong expression of Trk-B by MAH, predicting the biological responsiveness of MAH to BDNF and NT-4, and the expression of CNTF receptor in N18. Our results also suggest that CNTF is an autocrine factor for PC12 and MAH, since both lines express the growth factor as well as the receptor. Thus, RT-PCR is a valuable tool in growth factor research that can be used in complement to, and interactively with, other approaches such as bioassay, receptor binding, and immunochemical determination. It will be particularly useful for screening a large number of growth factors in minute areas of the brain in patients suffering from neurodegenerative diseases such as Parkinson's and Alzheimer's.

KEY WORDS: Reverse transcription-polymerase chain reaction; growth factors; neuronal cell line.

INTRODUCTION

Neurons are known to respond to a variety of growth factors, some of which are specific for neurons while others can affect non-neuronal cells as well. Likewise, neurons can secrete growth factors that may exert autocrine function or stimulate other cell types. The combinatorial and permutative relationships of these fac-

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Abbreviations: BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; GMF-β, glia maturation factor β; IGF-1, insulin-like growth factor 1: IGF-2, insulin-like growth factor 2; IL-1, interleukin 1; IL-6, interleukin 6; INS, insulin; LIF, leukemia inhibitory factor; NGF, nerve growth factor; NT-3, neurotrophin 3; NT-4, neurotrophin 4; PDGF, platelet-derived growth factor; RT-PCR, reverse transcription—polymerase chain reaction; SCF, stem cell factor; TGF-β, transforming growth factor β. "R" following a growth factor designates its receptor.

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tors makes possible the fine tuning of the neural network that is important for the adaptive and integrative behaviors of the organism. Recent molecular cloning and DNA sequencing of numerous growth factors along with their receptors provide an opportunity for the detection of their mRNAs by means of reverse transcription-polymerase chain reaction (RT-PCR). Because of the extreme sensitivity of the method, a large number of growth factors can be simultaneously studied in a limited sample size, eliminating variations usually arising from multi-sample comparisons. In a previous paper (51), we reported the use of RT-PCR to study the expression of growth factors in cultured glial cells. In this paper we conducted a similar study on three neuronal cell lines: the PC12 rat pheochromocytoma line, the MAH rat sympathoadrenal line, and the N18 mouse neuroblastoma line.

EXPERIMENTAL PROCEDURE

Cell Culture. PC12 rat pheochromocytoma cell line, originated from Greene and Tischler (13), was obtained from American Type Culture Collection and cultured in uncoated plastic culture plates in RPMI 1640 supplemented with 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum. MAH is a v-myc-immortalized sympathoadrenal progenitor cell line (4). It was a gift of S. J. Birren and D. J. Anderson (California Inst. of Tech.) and was grown in poly-L-lysine-coated plastic culture plates in L15-CO_2 medium with 10% heat-inactivated fetal bovine serum, 5μM dexamethasone, and other supplements as described (4). N18 mouse neuroblastoma cell line was provided by M. Nirenberg and grown in uncoated plastic culture plates in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. All cells were grown at 37°C in 5% CO_2, 95% air, and saturated humidity to near confluence before harvested for RT-PCR analysis.

RNA Isolation and RT-PCR. Total RNA was extracted from cultured cells by the acid guanidinium thiocyanate-phenol-chloroform method (6) using the proprietary RNAzol B solution (Tel-Test, Inc.). The integrity of the extracted RNA was verified with agarose gel electrophoresis, using the 18S and 28S ribosomal RNA as indicators.

Reverse transcription was conducted in a reaction volume of 40 μl containing 2 μg of total RNA, 1 μg random hexamer primers (pd $[N]_6$, Pharmacia), 20 units RNasin (Pharmacia), and 8 μl of a solution comprising 250 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 375 mM KCl and 50 mM dithiothreitol. After heating for 5 min at 68°C, the reaction was initiated by addition of 20 μl of dNTP mix (dATP, dCTP, dGTP and dTTP, 1 mM each) and 300 units of Maloney murine leukemia virus (MMLV) reverse transcriptase (Pharmacia). The reaction was allowed to proceed at 37°C for 90 min and finally terminated by boiling for 5 min.

Polymerase chain reaction was carried out essentially as previously described (52). The first strand cDNA (2 μl) was added to the PCR mixture in a total volume of 50 μl containing the following components: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$, 250 μM each dNTP, 10 μM TMAC (tetramethylammonium chloride), 0.2 μM each oligonucleotide primer (sense and antisense) and 2.5 units of Taq polymerase. PCR was carried out on a Perkin-Elmer Thermal Cycler. The initial template denaturation was conducted at 94°C for 4 min and was followed by the following: primer annealing at 52°C for 1 min; primer extension at 72°C for 2 min; denaturation at 94°C for 1 min. At the end of the 42nd cycle, the reaction mixture was kept at 72°C for 3 min and then brought to room temperature. Fifteen μl of the PCR product was electrophoretically analyzed on a 2% agarose gel containing ethidium bromide. All RT-PCR experiments were routinely controlled by conducting PCR without first doing a reverse transcription reaction.

For uniformity of comparison, all PCR experiments (except for actin standard) were conducted for 42 cycles. For most growth factors and receptors this was within the range of exponential progression, as pre-determined from several preliminary trials. Since PCR is sensitive to variations in pH and magnesium concentration, for each primer set and template cDNA we also pre-determined and optimized these two variables using an Invitrogen "PCR optimizer kit". As a result, all PCR were conducted at pH 8.3 and 3 mM $MgCl_2$ except for the following: Trk-A, Trk-B, Trk-C, PDGF-A, PDGF-B and EGF-R at pH 9.5 and 2 mM $MgCl_2$; PDGF-Rα, PDGF-Rβ and IGF-1-R at pH 9.0 and 2 mM $MgCl_2$; IL-6 and IL-6-R at pH 8.5 and 1.5 mM $MgCl_2$.

PCR Primers. Table I shows all the oligonucleotide primers used for the PCR reactions. Details of the choice of these primer pairs were discussed in our previous publication (51). In general, primers of approximately 20–30 nucleotides long having equal A/T and G/C contents were selected from different exons. All primer sequences were checked with the database in GenBank to avoid inadvertent homology with other proteins. Oligonucleotide primers were synthesized on an Applied Biosystems 394 DNA Synthesizer using standard phosphoramidite chemistry.

In all experiments, RT-PCR of actin was carried out concurrently (25 cycles) to control for sample loading. Primers were taken from rat and mouse β-actin according to Yamamori (48) as shown below, with a predicted product size of 285 bp:

Sense	5'-TCATGAAGTGTGACGTTGACATCCGTAAAG-3'
Antisense	5'CCTAGAAGCATTTGCGGTGCACGATGGAGG-3'

Sequencing of PCR Products. To verify authenticity of the PCR products, we sequenced all the amplified materials of expected molecular size. To this end the DNA bands of interest were excised from the agarose gel after electrophoresis and purified by the Geneclean procedure (B10-101, La Jolla, CA). The purified DNA was directly sequenced using Taq DNA polymerase in a cycling protocol with fluorescent dideoxynucleotide terminators (PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit, Applied Biosystems Division, Perkin Elmer, Foster City, CA). Reaction products were analyzed on an Applied Biosystems 373A DNA Sequencer. For authenticity check, published sequences were obtained from the GENBANK database (NCBI) using the MacDNASIS (Hitachi, San Bruno, CA) or Entrez (NCBI, Bethesda, MD). Comparisons of sequences of PCR products to published DNA sequences were performed using the Sequence Navigator program (Applied Biosystems).

RESULTS AND DISCUSSION

In this study cultured neuronal cells at near-confluent density were harvested and the total RNA extracted for RT-PCR for the detection of mRNAs of various growth factors and their receptors. The following three cell types were investigated: PC12 rat pheochromocytoma cell line, MAH rat sympathoadrenal line, and N18

Table I. PCR Primer Sequences for Growth Factors and Their Receptors

Growth Factor (or receptor)	Primer sequence (5'-3')	Predicted product size (bp)	Reference
NGF	GTT TTG GCC AGT GGT CGT GCA G		
	CCG CTT GCT CCT GTG AGT CCT G (h)	498	32
BDNF	ATG ACC ATC CTT TTC CTT ACT ATG GT		
	TCT TCC CCT TTT AAT GGT CAA TGT AC (h,r)	741	35
NT-3	GAA AAT TAC CAG AGC ACC CT		
	TGA CTC TTA TGC TCT GCA TA (r)	269	22
Trk A	CTG GGC GGA GTG CCT GAA		
	GGC TGC CGG CTC CAG GAA (h)	523	25
Trk B	AAT GAA ACA AGC CAC ACA CAG GGC		
	TGG AGT GTT ACT CCC ATT GGA GAT (r,m)	750	19
Trk C	AGC AAT GGG AAT GCC AGC ATC AAC		
	CTC CTT GAG GAA GTG GCC ATT (r,p)	1002	21
p75	GAG CCG TGC AAG CCG TGC ACC		
	CTC AGG CTC CTG GGT GCT GGG (h,r)	438	33
CNTF	TGG CTA GCA AGG AAG ATT CGT		
	ACG AAG GTC ATG GAT GGA CCT (h,r)	468	26
CNTF-R	CAT GGC CTG GAA CTG GGC CAC AGT		
	TGT CCC AAT CTC ATT GTC CTT GGC (h)	638	10
LIF	GAA AAC GGC CTG CAT CTA AGG		
	GCC ATT GAG CTG TGC CAG TTG (h)	240	45
LIF-R	GTC TGT GAG GGA AGC AGT GC		
	TCT AGG AGA GTC TGG AGA CAC (h,m)	478	11
IL-6	TGC TGG TGA CAA CCA CGG CC		
	GTA CTC CAG AAG ACC AGA GG (h)	308	45
IL-6-R	CTC AGC AAT GTT GTT TGT GAG		
	CGA TAT CTG AGC TCA AAC CGT (h)	394	36
gp130	CCT CAC ACT CCT CCA AGG CAC		
	CTG GAT TCA TGC TGA CTG CAG (h)	473	15
PDGF-A	CCT GCC CAT TCG GAG GAA GAG		
	TTG GCC ACC TTG ACG CTG CG (h)	225	3
PDGF-B	GAA GGA GCC TGG GTT CCC TG		
	TTT CTC ACC TGG ACA GGT CG (h)	217	3
PDGF-Rα	CAC CTG AGT GAG ATT GTG GAG		
	AGA GTC TAT GCC GAT GTC GTC (h,m)	408	8
PDGF-Rβ	TGA CCA CCC AGC CAT CCT TC		
	GAG GAG GTG TTG ACT TCA TTC (h,m)	228	8
bFGF	GCC TTC CCG CCC GGC CAC TTC AAG G		
	GCA CAC ACT CCT TTG ATA GAC ACA A (h)	179	1
bFGF-R	GAC GCA ACA GAG AAA GAC TTG T		
	GCC AGC AGT CCC GCA TCA TCA T (h)	661	44
EGF	ACA GCC CTG AAG TGG ATA GAG		
	GGG CTT CAG CAT GCT GCC TTG (h,m)	592	2
EGF-R	AGG TCT TGA AGG CTC TCC AAC		
	GTC TTT GTG TTC CCG GAC ATA (h)	526	41
TGF-β	GAA GCC ATC CGT GGC CAG AT		
	GAC GTC AAA AGA CAG CCA CT (h,r,m)	461	5
TGF-β-R	AAG TAC AGA TCA GGG ACC AGG		
	GAG CAG GTC GTA TGT CAA CTA (r)	578	43
GMF-β	CCG CTG ACG GCC GGA AGG		
	TTA TGT CTG GAT CCA GTA TGG TCA GGT (h,r)	512	50
SCF	GCC TTT CCT TAT GAA GAA GA		
	TGC AAC AGG GGG TAA CAT AAA TGG (h,r,m)	575	24
SCF-R	CGT TGA CTA TCA GTT CAG CGA G		
	CTA GGA ATG TGT AAG TGC CTC C (h)	360	49
IL-1	CAG TTC TGC CAT TGA CCA TCT		
	CCG ATG AGT AGG CAT ACA TGT (r)	466	30
IL-1-R	GAG GGA CAG TTT GGA TAC AAG		
	TGC CGG CAG TTT CTC CTT AGT (m)	452	37
IGF-1	ATG TCC TCC TCG CAT CTC TTC		
	CCT GTA GTT CTT GTT TCC TGC (h)	377	34
IGF-1-R	ATG GAG GAG AAG CCG ATG TGT		
	GTT CTC CAG CTC TGA AGC AAT (h)	572	40
IGF-2	AAG TCG ATG CTG GTG CTT CTC		

Table I. Continued

Growth Factor (or receptor)	Primer sequence (5'-3')	Predicted product size (bp)	Reference
	TTC CGA TTG CTG GCC ATC TCT (h)	520	34
IGF-2-R	CAT GGG AAG CTG TTG ATA CCA		
	CTC GTA GTG TGT CTA TGT CTC (h)	502	28
INS	ATG GCC CTG TGG ATG CGC		
	GTT GCA GTA GTT CTC CAG (h,r)	330	9
INS-R	AGG TTG CAT GAG CTG GAG AA		
	GAA CAG TTG CCC AGG CAC TC (h,r)	595	12

First primers are sense strands; second primers are antisense strands. Species of origin for primer sequences are indicated in parentheses following each primer pair (h = human, r = rat, m = mouse, p = porcine).

mouse neuroblastoma line. Fig. 1 is a composite photograph of the RT-PCR products of all the growth factors and receptors included in this study, as visualized on agarose gels after electrophoresis. The scores of band intensity of the products are summarized in Table II.

Among the neurotrophin family (20), our results show that PC12 is rich in Trk-A and p75, the high- and low-affinity receptors of NGF (25), in agreement with the well-known fact that PC12 is very responsive to NGF (13). On the other hand, MAH is rich in Trk-B, predicting its responsiveness to BDNF and NT-4, although we are not aware of any literature report to this effect. The lack of response of MAH to NGF in unprimed cultures as observed by Birren and Anderson (4) may be explained by the low level of Trk-A in these cells. None of the neuronal lines express any trace of neurotrophins, suggesting that the neurotrophins are not autocrine factors for these neurons under investigation.

Among the neuropoietic cytokine family (20,31) which includes CNTF, LIF, IL-6 and oncostatin M, we found that both CNTF and its receptor complex are expressed by PC12 and MAH. The CNTF receptor complex comprises CNTF-R, LIF-R, and gpl30 (38). Thus, our data not only provide a molecular basis for the observations that PC12 (52) and MAH (17) are responsive to CNTF, but also suggest that CNTF could be an autocrine growth factor for both cell lines. On the other hand, we observed that N18 expresses high levels of LIF-R and gpl30, the two components of the LIF receptor complex (38), predicting its responsiveness to LIF. However, LIF is probably not an autocrine factor for N18 because the cell line does not express LIF. None of the three neuronal lines express IL-6 or IL-6 receptor.

Like the glial cultures, all the three neuronal lines express high levels of PDGF-A but not its receptor. MAH expresses bFGF-R, in agreement with a previous report that MAH responds to bFGF (4). However, our failure to detect bFGF-R in PC12 is at variance with some reports that PC12 is responsive to bFGF (39,42). This probably resulted from divergence in cell passage between laboratories since we also failed to detect a neurite-extension effect of bFGF on our PC12 line (results not shown). For the same reason, our RT-PCR did not detect the presence of EGF-R in our PC12 line, nor were we able to observe a mitogenic effect on these cells (14). Nevertheless, a technical problem cannot be ruled out for these negative results.

Fig. 1 and Table II also show that PC12 and N18, but not MAH, strongly express TGF-β while none express the receptor. On the other hand, all the three lines express GMF-β at a significant level, suggesting neuron-glia interaction. The lack of a GMF-β-R cDNA precluded our determination of the expression of the receptor. MAH expresses SCF-R, the receptor for a growth factor (SCF) known to stimulate the proliferation and maturation of hematopoietic stem cells (47). This finding is in line with the reports that SCF-R is present in both adult and embryonic nervous system (16,18,23,27,29). However, the function of SCF on neurons has not been clearly defined. All the three lines express insulin receptor, a finding consistent with the known survival effect of insulin on neuronal cells. In general, neurons express a more limited and selective number of growth factors and/or receptors compared to glia.

To our knowledge, the current work is the most comprehensive exploration of the use of RT-PCR for the study of growth factors in neuronal cells. We have attained three distinct goals. Firstly, we have demonstrated the reliability of RT-PCR for detecting growth factors and their receptors by confirming the existing knowledge on neurons, such as the presence of neurotrophin receptors. Secondly, we have obtained new information that can expand and extend what was previously known about neurons. For example, we have for the first time presented evidence on the presence of CNTF receptor

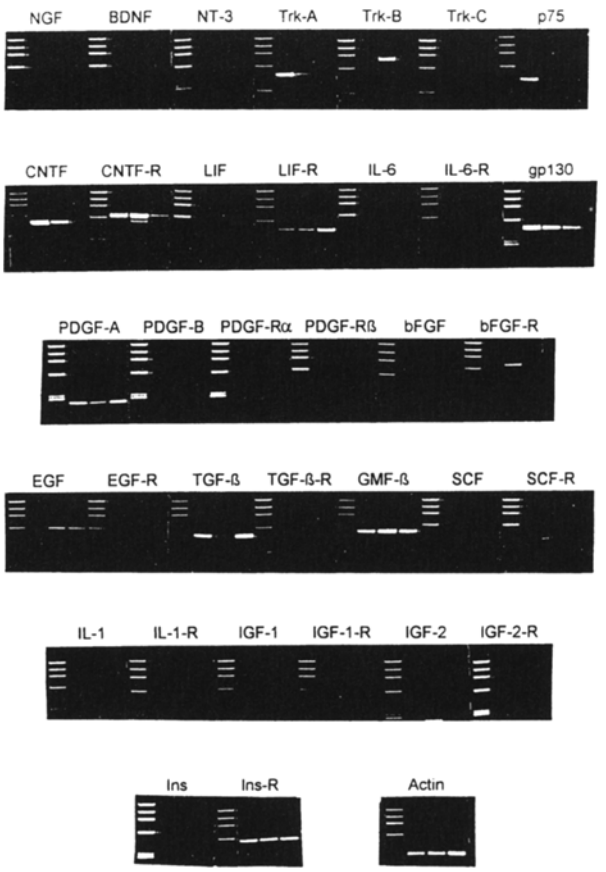

Fig. 1. Composite photograph showing RT-PCR products from mRNAs of various growth factors and receptors in PC12, MAH and N18 neuronal cell lines. Equivalent amounts of total RNA were used as starting material, and RT-PCR was carried out according to the procedure described in METHODS. The products were electrophoresed in 2% agarose gel stained with ethidium bromide and photographed under ultraviolet light. Each small photograph contains a size ladder (first lane) and samples from PC12 (second lane), MAH (third lane) and N18 (fourth lane). The size standards are (top to bottom): 1353 bp, 1078 bp, 872 bp, 603 pb, 310 bp. Growth factors or receptors to be studied are indicated above each photograph. All experiments were repeated several times on different batches of cultured cells, and the results presented are those that are reproducible in at least three cultures. A weak, false positive band (wrong size product) is seen in MAH for Trk-C.

Table II. Relative Band Intensity of RT-PCR Products

Growth Factor (or receptor)	PC12	MAH	N18
NGF	–	–	–
BDNF	–	–	–
NT-3	–	–	–
Trk-A	++++	+	±
Trk-B	+	+++	–
Trk-C	–	–	–
p75	++	–	–
CNTF	++++	++	–
CNTF-R	++++	++++	+
LIF	–	–	–
LIF-R	+	+	+++
IL-6	–	–	–
IL-6-R	–	–	–
gp130	++++	+++	++
PDGF-A	+++	++	+++
PDGF-B	–	–	–
PDGF-Rα	–	–	–
PDGF-Rβ	–	–	–
bFGF	–	–	–
bFGF-R	–	++	–
EGF	±	++	+
EGF-R	–	–	–
TFG-β	+++	±	++++
TFG-β-R	–	–	–
GMF-β	+++	++++	+++
SCF	±	±	–
SCF-R	–	++	–
IL-1	–	–	–
IL-1-R	–	–	–
IGF-1	–	–	–
IGF-1-R	–	–	–
IGF-2	±	±	–
IGF-2-R	–	–	–
INS	–	–	–
INS-R	++	++	++

Results in Fig. 1 were scored as follows: (–) absent, (+) low abundance, (++) moderate abundance, (+++) high abundance, (++++) very high abundance. Products with at least one "+" in any cell type were sequenced. When more than one cell type showed positive results, the sequence was obtained from the most positive one. All such products yielded definitive sequences with at least 95% indentity matched to the respective published sequences.

complex in PC12, a finding that explains our earlier report that CNTF enhances the biological effects of NGF on PC12 (52). Thirdly, we have obtained novel information that can lead the way to further experiments using the more conventional methods such as bioassay, receptor binding, and immunochemical determination. This is exemplified by the expression of Trk-B in MAH, suggesting that this cell line is responsive to BDNF and NT-4.

Lastly, one should understand the inherent strength and limitations of RT-PCR. The enormous amplification power of PCR makes it feasible to analyze well over 50 different proteins simultaneously from a few thousand cells or a few milligrams of wet tissue. When applied in a clinical setting, one can easily screen all the known growth factors and their receptors from very discrete areas of the human brain. False positive reactions can be ruled out by proper controls (such as omitting the RT step) and by sequencing the PCR products. However, one should be aware of the fact that, although it can usually be assumed that the synthesis of a protein follows the expression of its mRNA, there are rare instances where this is not so. On the other hand, a negative reaction could mean either the absence of the mRNA or the presence of a technical problem. Thus, a

final conclusion on protein expression (rather than mRNA expression) should await immunologic confirmation such as western blot. But since it is much easier to synthesize multiple PCR primers than to produce a large number of specific antibodies, the discretional use of RT-PCR for the study of mRNA is a logical first step for the simultaneous screening of the genetic expression of multiple growth factors (and their receptors) from a very limited sample size. The promise of this approach in pathologic studies of the brain as in Parkinson's or Alzheimer's disease and brain tumors is obvious from the current study.

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REFERENCES

1. Abraham, J. A., Whang, J. L., Tumolo, A., Mergia, A., Friedman, J., Gospodarowicz, D., and Fiddes, J. C. 1986. Human basic fibroblast growth factor: nucleotide sequence and genomic organization. EMBO J. 5:2523–2528.
2. Bell, G. I., Fong, N. M., Stempien, M. M., Wormsted, M. A., Caput, D., Ku, L., Urdea, M. S., Rall, L. B. and Sanchez-Pescador, R. 1986. Human epidermal growth factor: cDNA sequence, expression in vitro and gene organization. Nucleic acids Res. 14: 8427–8446.
3. Betsholtz, C., Johnsson, A., Helding, C-H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, A. L., Knott, T. J., and Scott, J. 1986. cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumor cell lines. Nature 320:695–699.
4. Birren, S. J., and Anderson, D. J. 1990. A v-myc-immortalized sympathoadrenal progenitor cell line in which neuronal differentiation is initiated by FGF but not NGF. Neuron 4:189–201.
5. Burt, D. W., and Paton, I. R. 1992. Evolutionary origins of the transforming growth factor-beta gene family. DNA and Cell Biology 11:497–510.
6. Chomczynski P., and Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocynate-phenol-chloroform extraction. Anal. Biochem. 162:156–159.
7. Claesson-Welsh, L., Ericksson, A., Moren, A., Severinsson, L., Ek, B., Ostman, A., Betsholtz, C., and Heldin, C-H. 1988. cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. Mol. Cell Biol. 8:3476–3486.
8. Claesson-Welsh, L., Ericksson, A., Westermark, B., and Heldin, C-H. 1989. cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. Proc. Natl. Acad. Sci. USA 86:4917–4921.
9. Cordell, B., Bell, G., Tischer, E., DeNoto, F. M., Ullrich, A., Pictet, R., Rutter, W. J., and Goodman, H. M. 1979. Isolation and characterization of a cloned rat insulin gene. Cell 18:533–543.
10. Davis, S., Aldrich, T. H., Valenzuela, D. M., Wong, V., Furth, M. E., Squinto, S. P., and Yancopoulos, G. D. 1991. The receptor for ciliary neurotrophic factor. Science 253:59–63.
11. Gearing, D. P., Thut, C. J., VandenBos, T., Gimpel, S. D., Delaney, P. B., King, J., Price, V., Cosman, D., and Beckmann, M. P. 1991. Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. EMBO J. 10:2839–2848.
12. Goldstein, B. J., and Dudley, A. L. 1990. The rat insulin receptor: primary structure and conservation of tissue-specific alternative messenger RNA splicing. Mol. Endocrinology 4:235–244.
13. Greene, L. A., and Tischler A. S. 1976. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. U.S.A. 73:2424–2428.
14. Guroff G., Dickens G., and End D. 1981. The induction of ornithine decarboxylase by nerve growth factor and epidermal growth factor in PC12 cells. J. Neurochem. 37:342–349.
15. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T., and Kishimoto, T. 1990. Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63:1149–1157.
16. Hirota, S., Ito, A., Morii, E., Wanaka, A., Tohyama, M., Kitamura, Y., and Nomura, S. 1992. Localization of mRNA for c-kit receptor and its ligand in the brain of adult rats. Mol. Brain Res. 15:47–54.
17. Ip, N. Y., Boulton T. G., Li Y., Verdi J. M., Birren S. J., Anderson D. J., and Yancopoulos G. D. 1994. CNTF, FGF and NGF collaborate to drive the terminal differentiation of MAH cells into postmitotic neurons. Neuron 13:443–455.
18. Keshet, E., Lyman, S. D., Williams, D. E., Anderson, D. M., Jenkins, N. A., Copeland, N. G., and Parada, L. F. 1991. Embryonic RNA expression patterns of the c-kit receptor and its cognate ligand suggest multiple functional roles in mouse development. EMBO J. 10:2425–2435.
19. Klein, R., Parada, L. F., Coulier, F., and Barbacid, M. 1989. TrkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. EMBO J. 8:3701–3709.
20. Korsching, S. 1993. The neurotrophic factor concept: a reexamination. J. Neurosci. 13:2739–2748.
21. Lamballe, F., Klein, R., and Barbacid, M. 1991. Trk C, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell 66:967–979.
22. Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M., and Yancopoulos, G. D. 1990. Neurotrophin-3: A neurotrophic factor related to NGF and BDNF. Science 247:1446–1451.
23. Manova, K., Bachvarova, R. F., Huang, E. J., Sanchez, S., Pronovost, S. M., Velazquez, E., McGuire, B., and Besmer, P. 1992. c-kit receptor and ligand expression in postnatal development of the mouse cerebellum suggests a function for c-kit in inhibitory neurons. J. Neurosci. 12:4663–4676.
24. Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., Mcniece, I. K., Jacobson, F. W., Mendiaz, E. A., Birkett, N. C., Smith, K. A., Johnson, M. J., Parker, V. P., Flores, J. C., Patel, A. C., Fisher, E. F., Erjavec, H. O., Herrera, C. J., Wypych, J., Sachdev, R. K., Pope, J. A., Leslie, I., Wen, D., Lin, C-H., Cupples, R. L., and Zsebo, K. M. 1990. Primary structure and functional expression of rat and human stem cell factor DNAs. Cell 63:203–211.
25. Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T., and Barbacid, M. 1989. Molecular and biochemical characterization of the human trk proto-oncogene. Mol. Cell. Biol. 9:24–33.
26. Masiakowski, P., Liu, H., Radziejewski, C., Lottspeich, F., Oberthuer, W., Wong, V., Lindsay, R. M., Furth, M. E., and Panayotatos, M. 1991. Recombinant human and rat ciliary neurotrophic factors. J. Neurochem. 57:1003–1012.

27. Matsui, Y., Zsebo, K., Hogan, B. L. 1990. Embryonic expression of a haematopoietic growth factor encoded by the S1 locus and the ligand for c-kit. Nature 347:667–669.
28. Morgan, D. O., Edman, J. C., Standring, D. N., Fried, V. A., Smith, M. C., Roth, R. A., and Rutter, W. J. 1987. Insulin-like growth factor II receptor as a multifunctional binding protein. Nature 329:301–307.
29. Natali, P. G., Nicotra, M. R., Sures, I., Santoro, E., Bigotti, A., and Ullrich, A. 1992. Expression of c-kit receptor in normal and transformed human nonlymphoid tissues. Cancer Res. 52:6139–6143.
30. Nishida, T., Nishino, N., Takano, M., Sekiguchi, Y., Kawai, K., Mizuno, K. U., Nakai, S., Masui, Y., and Hirai, Y. 1989. Molecular cloning and expression of rat interleukin-1 alpha cDNA. J. Biochem. 105:351–357.
31. Patterson, P. H. 1992. The emerging neuropoietic cytokine family. Current Opinion in Neurobiology 2:94–97.
32. Pizzuti, A., Borsani, G., Falini, A., Rugarli, E. I., Sidoli, A., Baralle, F. E., Scarlato, G., and Silani, V. 1990. Detection of b-nerve growth factor mRNA in the human fetal brain. Brain Research 518:337–341.
33. Radeke, M. J., Misko, T. P., Hsu, C., Herzenberg, L. A., and Shooter, E. M. 1987. Gene transfer and molecular cloning of the rat nerve growth factor receptor. Nature 325:593–597.
34. Rall, L. B., Scott, J., and Bell, G. I. 1987. Human insulin-like growth factor I and II messenger RNA: isolation of complementary DNA and analysis of expression. Methods Enzym. 146:239–248.
35. Rosenthal, A., Goeddel, D. V., Nguyen, T., Martin, E., Burton, L. E., Shih, A., Laramee, G. R., Wurm, F., Mason, A., Nikolics, K., and Winslow, J. W. 1991. Primary structure and biological activity of human brain-derived neurotrophic factor. Endocrinology 129: 1289–1294.
36. Scooltink, H., Stoyan, T., Lenz, D., Schmitz, H., Hirano, T., Kishimoto, T., Heinrich, P. C., and Rose-Jhon, S. 1991. Structural and functional studies on the human hepatic interleukin-6 receptor. Molecular cloning and over expression in Hep G2 cells. Biochem. J. 277:659–664.
37. Sims, J. E., March, C. J., Cosman, D., Widmer, M. B., Robson MacDonald, H., McMahan, C. J., Grubin, C. E., Wignal, J. M., Jackson, J. L., Call, S. M., Friend, D., Alpert, A. R., Gillis, S., Urdal, D. L., and Dower, S. K. 1988. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 241:585–589.
38. Taga, T., and Kishimoto, T. 1992. Cytokine receptors and signal transduction. FASEB J. 6:3387–3396.
39. Togari, A., Dickens G., Kuzuya H., and Guroff G. 1985. The effect of fibroblast growth factor on PC12 cells. J. Neurosci. 5: 307–316.
40. Ullrich, A. 1991. Insulin-like growth factor I receptor cDNA cloning. Methods Enzym. 198:17–26.
41. Ullrich, A, Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D., and Seeburg, P. H. 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 309:418–425.
42. Unsicker, K., Grothe, C., Otto, D., and Westermann, R. 1991. Basic fibroblast growth factor in neurons and its putative functions. Ann. NY Acad. Sci. 638:300–305.
43. Wang, X-F., Lin, H. Y., Ng-Eaton, E., Downward, J., Lodish, H. F., and Weinberg, R. A. 1991. Expression, cloning and characterization of the TGF-beta Type III receptor. Cell 67:797–805.
44. Wennstrom, S., Sandstrom, C., and Claesson-Welsh, L. 1991. cDNA cloning and expression of a human FGF receptor which binds acidic and basic FGF. Growth Factors 4:197–208.
45. Wesselingh, S. L., Gough, N. M., Finlay-Jones, J. J., and McDonald, P. J. 1991. Detection of cytokine mRNA in astrocyte cultures using the polymerase chain reaction. Lymphokine Research 9:177–185.
46. Westermark, R., Grothe, C., and Unsicker, K. 1990. Basic fibroblast growth factor (bFGF), a multifunctional growth factor for neuroectodermal cells. J. Cell Science 13 Suppl., 97–117.
47. Witte, O. N. 1990. Steel locus defines new multipotent growth factor. Cell 63:5–6.
48. Yamamori, T. 1991. Localization of cholinergic differentiation factor/leukemia inhibitory factor mRNA in the rat brain and peripheral tissues. Proc. Natl. Acad. Sci. USA 88:7298–7302.
49. Yarden, Y., Kuang, W-J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T. J., Chen, E., Schlessinger, J., Francke, U., and Ullrich, A. 1987. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J. 6: 3341–3351.
50. Zaheer, A., Fink, B., and Lim, R. 1993. Expression of glia maturation factor β mRNA and protein in rat organs and cells. J. Neurochem. 60:914–920.
51. Zaheer, A., Zhong, W., Uc, E. Y., Moser, D. R., and Lim, R. 1995. Expression of mRNAs of multiple growth factors and receptors by astrocytes and glioma cells: detection by RT-PCR. Cell. & Mol. Neurobiology, 15:221–237.
52. Zhong, W., Zaheer, A., and Lim, R. 1994. Ciliary neurotrophic factor (CNTF) enhances the effects of nerve growth factor on PC12 cells. Brain Res. 661:56–62.