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Modulation of fibroblast growth factor receptor expression and signalling during retinoic acid-induced differentiation of Tera-2 teratocarcinoma cells

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We have analyzed the regulation of fibroblast growth factor receptors (FGFRs) during retinoic acid (RA) induced differentiation of Tera-2 human embryonal carcinoma cells. Undifferentiated Tera-2 cells expressed mRNAs for all four known FGFRs. Their differentiation led to loss of FGFR-4 mRNA expression and mRNA levels for FGFR-2 and FGFR-3 were considerably downregulated, whereas the mRNA levels for FGFR-1 remained unaltered. A substantial decrease in binding of K-FGF was found to occur upon RA-induced differentiation of the cells. In undifferentiated Tera-2 cells FGF stimulation caused an increase of c-*fos* mRNA, and c-*jun* mRNAs, but no increase of *jun*B mRNA, whereas in the differentiated cells, FGFs strongly stimulated the expression of all three genes. Thus differentiation of the Tera-2 cells leads to marked changes in FGFR gene expression as well as to complex alterations in their responses to exogenous FGFs.

Members of the fibroblast growth factor (FGF) family have been implicated in morphogenesis and angiogenesis as well as growth of malignant tumors (1,2,3). Acidic and basic FGFs (aFGF and bFGF) stimulate the growth of mesoderm- and neuroectoderm-derived cells (4,5,6). Genes encoding the FGF homologues K-FGF and *int*-2 were originaly isolated as oncogenes (7,8,9). These genes have subsequently been shown to have specific expression patterns during embryogenesis suggesting a potential regulatory role during development (10,11).

The four known human FGF receptors (FGFRs) are encoded by a receptor tyrosine kinase gene family (12,13,14,15,16). FGFR1 and FGFR-2 have been shown to bind aFGF and bFGF equally well, whereas FGFR-4 preferentially binds aFGF (12,13,14,16,17). We have shown that these receptors have distinct expression patterns in human fetal tissues and specifically, the FGFR-4 mRNA was not present in developing brains from 17-18 week human fetuses, in contrast to the FGFR-1 (*flg*), FGFR-2 (*bek*) and FGFR-3 mRNAs (16). When these FGFs bind to their receptors, they activate genetic programs that lead to a readjustment of gene expression in the responding cells. Among the first set of genes activated are transcription factor genes c-*jun*, *jun*B and c-*fos* (18,19).

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The undifferentiated stem cells of teratocarcinoma tumors are called embryonal carcinoma (EC) cells. Many EC cells can be induced to differentiate *in vitro*, and have been used as models for cell differentiation during early embryonic development (20,21). The differentiation of EC cells leads to changes in the endogenous FGF-like activity and in the binding of FGFs (22,23,24). When differentiated with retinoic acid (RA), the human Tera-2 EC cells convert from rapidly growing undifferentiated cells into a slowly proliferating phenotype associated with the expression of neuronal markers (25,26,27). This process is also associated with a decrease of plasminogen activator activity produced by the Tera-2 cells as well as modulation of their proteolytic responses to exogenous bFGF (28).

RA-induced differentiation of Tera-2 cells leads to a decreased expression of K-FGF (29,30,31). Studies on a closely related cell line NTera-2 suggest that the differentiation process also leads to a decrease in expression of bFGF (22,30). Since autocrine or paracrine mechanisms may be involved in the regulation of the undifferentiated vs. differentiated phenotype of teratocarcinoma cells we have studied whether differentiation of Tera-2 cells would lead to changes in the binding of FGFs or in the expression of FGF-receptors or in the responses of the cells to exogenous FGFs.

MATERIALS AND METHODS

Cell culture. Tera-2 cells of clone 13 were provided by Dr C. F. Graham (Department of Zoology, University of Oxford, UK). Cells between passages 18-40 were used in this study. The cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum and antibiotics. To induce differentiation, the cells were plated on gelatin-coated tissue-culture grade dishes at a density of $1{,}5 \times 10^3$ cells/cm^2. On the following day, 5×10^{-6} M RA was added to the medium. The cells were cultured in the presence of RA for up to 10 days. To study FGF responses in undifferentiated and differentiated Tera-2 cells, FGFs were added to the culture medium at the concentration of 10 ng/ml for 1 h or 12 h before mRNA extraction.

Thymidine incorporation assay. The cells were labeled with 1 μCi/ml [^{3}H]-thymidine for 3 h, washed with PBS and precipitated with 10% TCA. They were then solubilized in 0.5 N NaOH containing 1% SDS and the solubilized radioactivity was processed for scintillation counting.

Growth factors and reagents. All-trans RA was from Sigma (St. Louis, MO). Recombinant bFGF was from Boehringer Mannheim (Mannheim, Germany). K-FGF was kindly provided by Dr. Claudio Basilico (New York University Medical Center, NY, USA) and aFGF by Dr. Michael Jaye (Rorer Central Research, King of Prussia, PA, USA).

FGF-receptor crosslinking. Labeling of FGFs was done according to the Chloramine-T method (32). The specific activities were 100 000-200 000 cpm/ng growth factor. Confluent, undifferentiated and RA-treated Tera-2 cells in 90 mm diameter culture dishes were washed twice with a binding buffer (DMEM containing 0.1% gelatin and 50 mM HEPES, pH 7.5). About 25 ng of [^{125}I]-FGF in the binding buffer was added on ice and incubation continued for 90 min. The cells were washed once with the binding buffer, twice with PBS and incubated at 4 °C in PBS containing 0.3 mM of the covalent crosslinker disuccidimidyl suberate for 20 min. The cells were then washed once with 10 mM HEPES, pH 7.5, 200 mM glycine, 2 mM EDTA and once with PBS, lysed in a lysis buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1.5 mM $MgCl_2$, 1.0 mM EDTA, 1 mg/ml Aprotinin) and centrifuged for 10 min at 10 000 x g. Aliquots of the supernatants were analysed in SDS-PAGE.

RNA extraction and analysis. Polyadenylated RNA was extracted from 10^7-10^8 cells at various time points after RA and FGF induction. Poly(A)$^+$ mRNA was bound to oligo-dT cellulose directly from cell lysates and eluted as described (33). 4 μg of RNA was dissolved in sample buffer, size-fractionated in formaldehyde-agarose (0.8%) gels and transferred to Biodyne membranes (Pall Corporation, Glen Cove, N.Y.) in 20xSSC (1x SSC is 150mM NaCl, 15 mM sodium acetate, pH 7.0) for 20 h. Hybridizations and washes were done in standard high-stringency conditions. The filters were exposed to Kodak XAR-5 film at -70 °C for 6-48 h. Radioactive signals were quantitated from the autoradiograms with a densitometric scanner (Helena Laboratories, Beaumont, TX).

Molecular probes. The FGFR-3 and FGFR-4 cDNAs have been described by Partanen et al. (16). The following cDNA clones were kindly given to us by the following investigators: bFGF, Andreas Sommer (34); K-FGF/*hst*-1, Masaaki Terada (7); c-*fos*; Päivi Koskinen (unpublished); *jun*B, Daniel Nathans (35); c-*jun*, Peter Angel (36); *flg* (37,38) and *bek* (13) Craig Dionne and Michael Jaye; GAPDH, Philippe Fort (39). Radioactive labeling was done by the random priming method (40).

RESULTS

Expression of FGF mRNAs undifferentiated and differentiated Tera-2 cells. For induction of differentiation of the Tera-2 cells they were grown in the presence of RA for 10 days. To verify that FGF mRNAs in our differentiation system were regulated similarly to what has been described earlier in differentiating human EC cells (29,30,31), polyadenylated RNA was isolated from the cells and subjected to Northern blotting and hybridization analysis with FGF-specific probes. Undifferentiated Tera-2 cells were found to express mRNA for bFGF and K-FGF, but no aFGF, *int*-2 or KGF mRNA. The RA-differentiated cells had lost the K-FGF mRNA and the bFGF mRNA was downregulated by about 90% (data not shown).

Affinity labeling of FGF receptors in undifferentiated and differentiated Tera-2 cells. The binding of FGFs to Tera-2 cell surface receptors was studied by incubating the cells with [^{125}I]-bFGF, [^{125}I]-aFGF or [^{125}I]-K-FGF. After washing to remove the unbound ligand the cells were treated with the covalent crosslinker disuccidimidyl suberate, dissolved and analyzed by SDS-PAGE and autoradiography. NIH 3T3 cells transfected with FGFR-1 or FGFR-4 were used for comparison. When labeled with [^{125}I]-bFGF, [^{125}I]-aFGF or [^{125}I]-K-FGF, the undifferentiated Tera-2 cells showed a receptor band of 150 kD which comigrated with the FGFR-1 from transfected NIH 3T3 cells (41) (**Fig. 1A**). This band was completely competed off by a 50-fold excess of nonradioactive aFGF (data not shown). No bands were seen in the molecular weight range of FGFR-4. Crosslinking with [^{125}I]-bFGF or [^{125}I]-aFGF showed the 150 kD band in cells treated for 10 d with RA, but the intensity of binding was slightly reduced. On the other hand, RA-treated cells crosslinked with [^{125}I]-K-FGF had only 10% of the radioactivity seen in the 150 kD band of untreated cells (**Fig. 1A**). Similar

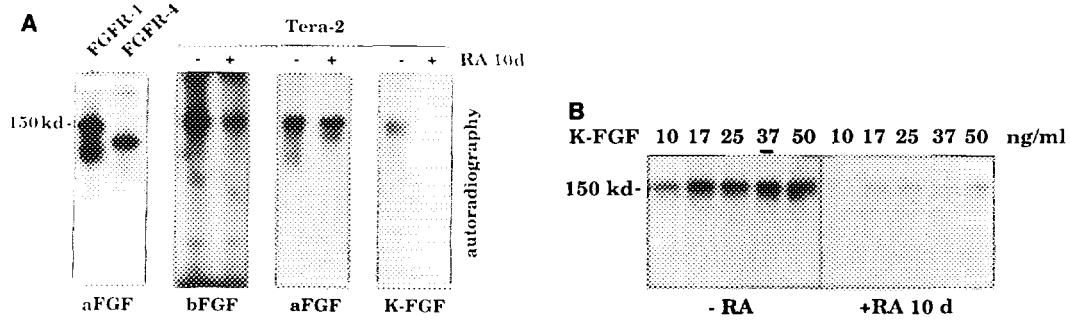
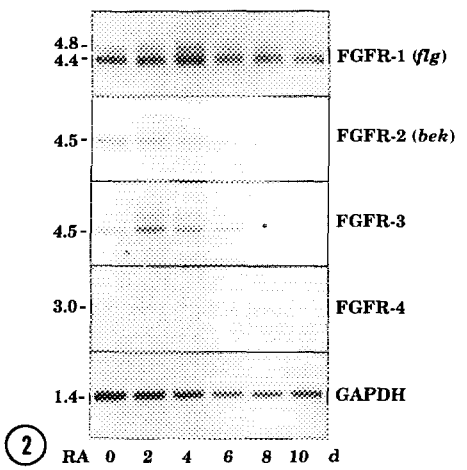


Fig. 1. Comparison of FGF receptors of undifferentiated and differentiated Tera-2 cells. (**A**) The first panel shows aFGF-crosslinked polypeptides from NIH 3T3 cells overexpressing transfected FGFR-1 and FGFR-4 cDNAs. The FGFR-1 polypeptides migrate at m.w. 150 000. The three following panels show crosslinking of bFGF, aFGF and K-FGF to undifferentiated (-RA) and differentiated (+RA) Tera-2 cells. In (**B**), shown is a titration crosslinking experiment with different concentrations of K-FGF. As can be seen from the figure, saturation of the receptors is obtained with concentrations above 17 ng/ml in both cells, but a similar difference in the amount of radioactive ligand-crosslinked receptor persists with these concentrations.

results were obtained with 10-50 ng/ml of labelled K-FGF, indicating that saturating amounts of ligand were used in the comparison (**Fig. 1B**). Differentiation of Tera-2 cells thus resulted in a significant decrease of specific K-FGF binding.

Regulation of FGFR mRNAs during the differentiation of Tera-2 cells. Because of the decreased binding of FGFs to differentiated Tera-2 cells we analysed possible changes in their expression of FGFR1-4 mRNAs upon RA-induced differentiation. As can be seen from **Fig. 2**, undifferentiated Tera-2 cells contained mRNAs for all four known FGFRs, although FGFR-1 mRNA was the predominant species. The FGFR-1 mRNA was not significantly downregulated during 10 days of RA treatment, whereas FGFR-2 mRNA was decreased by about 90% and FGFR-4 disappeared completely during this time period. FGFR-3 mRNA was initially somewhat increased, but subsequently downregulated by about 80%. As in the case of undifferentiated Tera-2 cells, bFGF treatment caused a further downregulation of FGFR-3 mRNA in the differentiated cells, but had no effect on the other receptor mRNAs (data not shown). No change of FGFR expression was seen when the undifferentiated cells were shifted to serum-free medium (data not shown), showing that the effects were not due to a slowdown of cell growth.

FGF regulation of *c-fos*, *c-jun* and *junB* mRNAs in undifferentiated and differentiated Tera-2 cells. Differentiation of Tera-2 cells resulted in profound changes in their FGFR mRNA expression. We therefore studied whether some of the immediate early transcription factor mRNAs would be differently regulated by exogenous FGFs in undifferentiated and differentiated Tera-2 cells. Northern blots were hybridized with the *fos* and *jun* transcription factor-specific probes. The differentiation-associated decrease of FGFR-2 mRNA was used as an internal control for FGFR downregulation by RA. In undifferentiated cells faint c-*jun* and c-*fos* signals were seen but no apparent



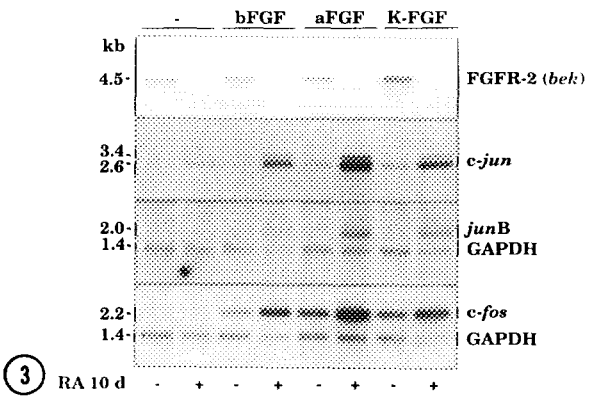


Fig. 2. Analysis of FGFR mRNAs in Tera-2 cells. Tera-2 cells were treated with RA for indicated times. Polyadenylated RNA from cells was isolated and analysed by Northern blotting and hybridization with the FGFR probes, as shown, and, as a control, with the GAPDH probe.

Fig. 3. Changes in the expression and FGF inducibility of immediate early mRNAs during Tera-2 cell differentiation. Undifferentiated and differentiated cells were treated for 1 h with the FGFs shown and analysed as in Fig. 2 using the c-*jun*, *junB* and c-*fos* probes. As a control for receptor downregulation, the FGFR-2 probe was used (uppermost panel).

*jun*B mRNA (**Fig. 3**). Stimulation with any of the three FGFs for 1 hour resulted in a transient induction of c-*fos* and about two fold induction of c-*jun* mRNA but did not induce *jun*B mRNA. In contrast, all three transcription factor mRNAs were prominently induced by FGFs in the differentiated Tera-2 cells. Thus even though in affinity labeling the differentiated cells showed markedly reduced binding of K-FGF, the cells showed a distinct response when stimulated with this growth factor.

FGF regulation of DNA synthesis. Because of the differences in the induction of immediate early growth factor-regulated mRNAs in the undifferentiated and differentiated cells, we were interested in comparing FGF induced DNA synthesis in these cells. When the undifferentiated cells were transferred to a medium containing 0.5 % serum DNA synthesis as measured by thymidine incorporation was decreased and there was very little difference in the between the wells incubated for 18 h with or without bFGF (**Fig. 4A**). In the differentiated cells bFGF was as potent as 10% FCS in maintaining DNA synthesis, while K-FGF showed lesser activity (**Fig. 4B**).

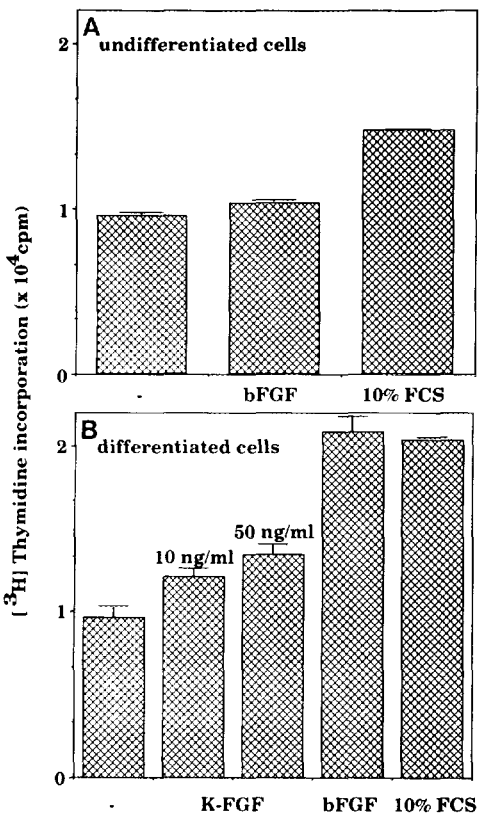


Fig. 4. Measurement of DNA synthesis in undifferentiated and differentiated Tera-2 cells stimulated with growth factors. The cells were changed to a growth medium containing 0.5% FCS and the growth factors indicated and thymidine incorporation was measured 18 h later as detailed in the Materials and Methods.

DISCUSSION

The present results show that undifferentiated Tera-2 cells express mRNAs for all four known FGF-receptors. Their RA-induced differentiation leads to a marked downregulation of FGFR-2 and FGFR-3 mRNAs and loss of transcripts for FGFR-4 while mRNA levels for FGFR-1 remain unchanged. These changes are associated with altered FGF regulation of immediate-early genes and DNA synthesis.

In addition to expression of FGFs, differentiation of Tera-2 cells also leads to changes in their binding of these factors. In the undifferentiated cells aFGF, bFGF and K-FGF were bound to polypeptides of 150 kD. These polypeptides migrate with the mobility of FGFRs 1-3 (13,14,15), which, like FGFR-4, were expressed at the mRNA level in the undifferentiated cells. None of the ligands, however, appeared to bind to a polypeptide corresponding to the mobility of FGFR-4. The mRNA levels for FGFR-4 were low, which is in line with the assumption that this receptor is not a major FGF binding protein in Tera-2 cells. Differentiation of the Tera-2 cells resulted in almost complete loss of binding of K-FGF. These changes relate to downregulation of FGFRs 2-3, observed at mRNA level. At present, FGFR-2 has the highest affinity for K-FGF among the known receptors (42). A strong downregulation of this receptor is therefore in line with a reduced binding of K-FGF, although specific antibodies for the various receptors are not yet available to prove this assumption.

In most cells, the c-*fos*, c-*jun* and *jun*B mRNAs as well as the AP-1 transcription factor are induced rapidly and transiently by different growth factors (43,44). The present results show that in the undifferentiated Tera-2 cells FGFs induce a small increase in the mRNA levels of c-*fos* and c-*jun* but *jun*B mRNA level was not induced. In the differentiated cells all three mRNAs were strongly induced. This altered FGF response probably reflects changes in mechanisms associated with regulation of immediate early genes in differentiating teratocarcinoma cells. In undifferentiated human Tera-2 cells and murine EC cells basal AP-1 activity is low, but it is greatly elevated in their differentiated derivatives (45).

In conclusion, the present results show that differentiation of Tera-2 cells leads to a modification in the FGF-signaling system leading to a complete loss of K-FGF and FGFR-4 gene expression as well as a strong downregulation in the expression of bFGF, FGFR-2 and FGFR-3 mRNAs. This is associated with a decrease in the binding of K-FGF. In the differentiated cells, K-FGF is also weaker growth factor than bFGF on basis of the relative abilities of these factors to maintain DNA synthesis of the cells. On the other hand, the mRNA levels for FGFR-1 remain unaltered and FGF induced AP-1 activity is greatly elevated in the differentiated cells.

Our results also show that the DNA synthesis of the undifferentiated Tera-2 cells is only marginally stimulated by exogenous FGF. This finding correlates with the failure of FGF to induce all immediate early mRNAs in these cells. One possiblity to explain these findings is that the undifferentiated cells produce enough FGFs to establish an autocrine stimulation of their own growth. This amount of FGF might not be sufficient to block all FGF receptors on the cells, since the cells could still be affinity labelled by radioiodinated FGFs. However, this possible autocrine stimulation might be sufficient to cause the downregulation of the responses of some immediate early genes. In the differentiated cells, less endogenous FGFs would be produced, which would result their observed slower growth rate, but, on the other hand, increased responses to exogenous FGFs. Thus, it is also possible that undifferentiated

teratocarcinoma cells are capable of stimulating selectively the growth of their differentiated derivatives by a paracrine growth factor mechanism.

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REFERENCES

1. Baird, A. and Klagsbrun, M. (1991) *Cancer Cells*, **3**, 239-243.
2. Rifkin, D. B. and Moscatelli, D. (1989) *J. Cell. Biol.*, **109**, 1-6.
3. Burgess, W. H. and Maciag, T. (1989) *Annu. Rev. Biochem.*, **58**, 575-606.
4. Walicke, P., Gowan, W., Uneo, N., Baird, A. and Guillemin, R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 3012-3016.
5. Thomas, K. A. and Gimenez-Gallego, G. (1986) *Trends. Biochem. Sci.*, **11**, 81-84.
6. Gospodarowicz, D. (1990) *In Current Topics in Developmental Biology*, vol. 24 (ed. M. Nilsen-Hamilton), pp. 57-93. Academic Press, San Diego, CA.
7. Taira, M, Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. and Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2980-2984.
8. Delli Bovi, P., Curatola, A., Kern, F., Greco, A., Ittman, M., and Basilico, C. (1987) *Cell*, **50**, 729-737.
9. Dickson, C. and Peters, G. (1987) Potential oncogene product related to growth factors. *Nature*, **326**, 833-833.
10. Jakobovits, A., Shackleford, G., Varmus, H. E. and Martin, G. R. (1986) *Proc. Natl. Acad. Sci. USA.*, **83**, 7806-7810.
11. Hebert, J., Basilico, C., Goldfarb, M., Huab, O. and Martin, G. R. (1990) *Dev. Biol.*, **138**, 454-463.
12. Johnson, D. E., Lee, P. L., Lu, J. and Williams, L. T. (1990) *Mol. Cell. Biol.*, **10**, 4728-4736.
13. Dionne, C. A., Crumley, G., Bellot, F., Kaplow, J. M., Searfoss, G., Ruta, M., Burgess, W. H., Jaye, M. and Schlessinger J. (1990) *EMBO J.*, **9**, 2685-2692.
14. Houssaint, E., Blanquet, P. R., Champion-Arnaud, P., Gesnel, M. C., Torriglia, A., Courtois, Y. and Breathnach, R. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 8180-8184.
15. Keegan, K., Johnson, D. E., Williams, L. T. and Hayman, M. J. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 1095-1099.
16. Partanen, J., Mäkelä, T., Eerola, E., Korhonen, J., Hirvonen, H., Claesson-Welsh, L. and Alitalo, K. (1991) *EMBO J.*, **10**, 1347-1354.
17. Vainikka, S., Partanen, J., Bellosta, P., Coulier, F., Basilico, C., Jaye, M. and Alitalo, K. (1992) *EMBO J.*, **11**, 4273-4280.
18. Almendral, J., Sommer, D., MacDonald-Bravo, H., Burckhard, J., Perera, J. and Bravo, R. (1988) *Mol. Cell. Biol.*, **8**, 2140-2148.
19. Ryder, K. and Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8464-8467.
20. Gardner, R. L. (1983) *Cancer Surveys*, Vol. **2**, No 1.
21. Andrews, P. (1988) *Biochim. Biophys. Acta*, **948**, 17-36.
22. Rizzino, A., Kuszynski, C., Ruff, E. and Tiesman, J. (1988) *Dev. Biol.*, **129**, 61-71.
23. Braunhut, S., Gudas, L., Kurokawa, T., Sasse, J. and D'Amore, P. (1989) *J. Cell Biol.*, **108**, 2467-2476.
24. Velcich, A, Delli-Dovi, P., Mantshukhani, A., Ziff, E. and Basilico, C. (1989) *Oncogene Res.*, **5**, 31-37.
25. Thompson, S., Stern, P. L., Webb, M., Walsh, F. S., Engström, W., Evans, E. P., Shi, W-K., Hopkins, B. and Graham, C. F. (1984). *J. Cell Sci.*, **72**, 37-64.
26. Webb, M., Graham, C. and Walsh, F. (1986) *J. Neuroimmunol.*, **11**, 67-86.
27. Mummery, C. L., van Rooijen, M. A., van den Brink, S. E. and de Laat, S. W. (1987) *Cell Differentiation*, **20**, 153-160.
28. Tienari, J., Alanko, T., Lehtonen, E. and Saksela, O. (1991) *Cell Regul.*, **2**, 285-297.
29. Tiesman, J. and Rizzino, A. (1989) *In Vitro Cell. Dev. Biol.*, **25**, 1193- 1198.

30. Miller, W. H. Jr., Moy, D., Li, A., Grippo, J. F. and Dmitrovsky, E. (1990) *Oncogene*, **5**, 511-517.
31. Schofield, P. N., Ekström, T. J., Granerus, M. and Engström, W. (1991) *FEBS Letters.*, **280**, 8-10.
32. Kan, M., Disorbo, D., Hou, J., Hoshi, H., Mansson, P-E. and Mckeehan, W. L. (1988) *J. Biol Chem.*, **263**, 11 306-11 313.
33. Sambrook, J., Fritsch, E., F. and Maniatis, T. (1989) Molecular cloning: A Laboratory manual. Cold Spring harbor Laboratory Press, Cold Spring Harbor NY.
34. Sommer, A., Brewer, M., T., Thompson, R. C., Moscatelli, D., Presta, M. and Rifkin, D. B. (1987) *Biohem. Bioph. Res. Comm.*, **144,** 543-550.
35.Lau, L. F. and Nathans, D. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1182-1186.
36. Angel, P., Allegretto, E., Okino, S., Hattori, K., Boyle, W., Hunter, T. and Karin, M. (1988) *Nature* **332**, 166-171.
37. Ruta, M., Howk, R., Ricca, G., Drohan, W., Zabelshansky, M., Laureys, G., Barton, D.E., Francke, U., Schlessinger, J. and Givol, D. (1988) *Oncogene*, **3**, 9-15.
38. Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger N., Kaplow, J., Crumley, G., Dionne, C., Jaye, M. and Schlessinger J. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8722-8726.
39. Forth, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. and Blanchard, J. M. (1985) *Nucl. Acids Res.*, **13**, 1431-1442.
40. Feinberg, A. P. and Vogelstein, B. (1984) *Anal. Biochem. addendum*, **137**, 266-267.
41. Wennström, S., Sandström, C. and Claesson-Welsh, L. (1991) *Growth Factors*, **4**, 197-208.
42. Mansukhani, A., Dell'era, P., Moscatelli, D., Kornbluth, S., Hanafusa, H. and Basilico, C. (1992) *Proc. Natl. Acad. Sci .USA*, **89**, 3305-3309.
43. Curran, T. and Franza, B. R. Jr. (1988) *Cell*, **55**, 395-397.
44. Bravo, R. (1990) *Cell Growth & Differentiation*, **1**, 305-309.
45. Wasylyk, C., Imler, J.L. and Wasylyk, B. (1988) *EMBO J.*, **8**, 2475-2483.*J. Cell. Biol.*, **108**, 2467-2476.