Onc 02

DIFFERENTIAL EXPRESSION OF THE c-src GENE DURING IN VITRO DIFFERENTIATION

Angelika Barnekow and Manfred Gessler

Acutely transforming retroviruses contain oncogenic sequences which are derived from counterparts in the normal cellular genome. Only little is known about the function of these cellular homologs termed c-onc genes. We have investigated the expression of c-src, the cellular counterpart of the Rous sarcoma virus transforming gene v-src. c-src is highly conserved during phylogenesis and appears first in the sponges (Barnekow and Schartl, Mol.Cell.Biol.4,1179,1984) The expression of the c-src gene was found to be tissue-specific and age-dependent(Barnekow and Bauer, BBA 782, 94,1984). Our data suggest that the physiological function of c-src appears to be more closely related to differentiation processes than to proliferation processes. This theory is supported by data obtained from experiments with the promyelocytic cell line HL-60. Induction of monocytic and granulocytic differentiation of HL-60 cells by 12-O-tetradecanoylphorbol-13-acetate(TPA) and dimethylsulfoxide (DMSO) is associated with an activation of the pp60$^{c\text{-}src}$ tyrosine kinase, but not with increased c-src gene expression. Control experiments exclude an interaction of TPA and DMSO themselves with the pp60$^{c\text{-}src}$ kinase. Using embryonal carcinoma cell lines, which also can be induced to differentiate in vitro, we are currently analyzing whether the differentiation-dependent expression of c-src is restricted to monomyelocytic cells or can be generally observed during cellular differentiation processes.

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Onc 03

HORMONAL REGULATION OF ONCOGENE EXPRESSION

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Insights into the hormonal control mechanisms of gene expression make it possible to subject genes to defined stimuli after in vitro recombination with specific regulatory sequences and reintroduction into the cellular context. The activated and normal human H-ras genes were subjected to the transcriptional regulation of the glucocorticoid responsive promoter region (LTR) of the mouse mammary tumor virus gene. Both chimeric genes are strictly dependent on hormone for their expression upon transfection into NIH 3T3 cells. Cells transfected with the LTR-H-ras genes were used to study the effect of oncogene and protooncogene expression on transformation parameters and on the transcription of cellular genes. H-ras oncogene induction in transfected cells results in phenotypic transformation, soft agar growth ability and tumorigenicity. The transcription from the MMTV LTR is adversely affected by the oncogene product accumulation and p21 production is down-regulated. The cellular c-myc, c-fos and ODC (ornithine decarboxylase) genes are enhanced in their expression by p21 accumulation. The normal H-ras protooncogene is not able to affect MMTV LTR transcription, and has only a slight effect on c-myc, c-fos and ODC expression. Both H-ras variants (oncogene and protooncogene) are, however, able to stimulate resting cells, synchronized by growth in low serum, to enter the S phase.
The activated H-ras oncogene was also subjected to the promoter region of the WAP (whey acidic protein) gene. This gene is regulated by lactogenic hormones and expressed in mammary epithelial tissue. Transgenic mice were derived which have stably acquired the WAP-ras gene in their genome. The tissue specificity and hormonal dependence of WAP expression was conferred to the H-ras gene. Tumors observed in these animals show a high level of WAP-ras expression, indicating a role for the transgene in in vivo tumorigenesis.

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Onc 04

THE ROLE OF ONCOGENES IN HUMAN TUMORS : A REVIEW

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Following the discovery of the first retroviral oncogene, v-src, and its cellular homologue, c-src, more than 20 different retroviral oncogenes (v-onc genes) and their normal cellular counterparts (proto-oncogenes, c-onc genes) have been identified, isolated and structurally analyzed in the past decade. In addition, an array of cellular oncogenes has been identified in, and isolated from, tumor DNA of various origins so that at present we know more than 30 different oncogenes and their normal homologues. Several lines of evidence suggest that c-onc genes are involved in human oncogenesis. (1) ras genes with specific point mutations have been detected in a wide variety of human tumor cells and tumors. In contrast to the normal ras proto-oncogenes, the point-mutated ras genes, upon introduction by DNA-mediated gene transfer techniques ('transfection') into mouse fibroblasts, induce foci of transformed cells in a monolayer of morphologically normal cells. The activation of the oncogenic potential of ras genes by point mutations seems to be associated with an impairment of an intrinsic GTPase activity.(2) The c-myc gene has been implicated in B-cell neoplasma because of a frequently observed reciprocal translocation with immunoglobulin genes. This rearrangement has been suggested to result in unregulated c-myc expression due to the loss of control elements. (3) The c-abl gene seems to play a role in human chronic myelogenous leukemia (CML), where it is translocated to chromosome 22 (Philadelphia Chromosome). As a consequence of this translocation, the c-abl gene product in CML cells possesses tyrosine protein kinase activity. (4) ras and myc genes have been found to be amplified in various tumor cells, and a variety of activated proto-oncogenes has been detected by the transfection assay in human tumours.

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TIm 01

BIOLOGICAL EFFECTS AND SPECIFIC MEMBRANE RECEPTORS FOR IFN-γ AND FOR TUMOR NECROSIS FACTOR ON HUMAN TUMOR CELLS

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Interferons and Tumor Necrosis Factor (TNF) are potentially effective antitumoral agents, which in in vitro models can exert synergistic activities. The effects of these cytokines are initiated by binding to specific high affinity membrane receptors (Kd ~100 pM), which are expressed on most cells of various tissue origins. Competition binding experiments suggest that distinct binding sites exist for IFN-γ, for IFN-α/-B and for TNF, respectively. Expression of IFN-γ receptors on both IFN-γ sensitive and on IFN-γ resistant cell lines and primary tumor cells clearly indicates that specific binding is a prerequisite, but is not sufficient to confer sensitivity to IFN-γ action. The same holds true for TNF sensitivity. Thus, both IFN-γ and TNF-resistance of given cells appears to be determined not only at the level of receptor expression, but also at a post-receptor level. Nevertheless, using HLA-DR inducible carcinoma cells, we have obtained evidence for a relationship between the quantity of IFN-γ receptors and the dose of IFN-γ required for induction of HLA-DR antigens, suggesting that under conditions of low concentrations of IFN-γ the number of expressed receptors might be response limiting in a priori sensitive tumor cells. As for hematopoietic cells, a great difference was found in receptor number between malignant and normal cells, determination of IFN-γ receptors may not only be important to evaluate potential effectivity of IFN-γ treatment, but may also be useful as an additional diagnostic parameter. Characterization of membrane receptors for IFN- and for TNF by DSS-crosslinking of receptor bound radiolabelled ligands suggest an IFN receptor molecule with an apparent molecular weight of ~130 kDa, consisting of two subunits and a single TNF receptor protein with an apparent molecular weight of ~76 kDa.

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