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THE p53 SUPPRESSOR GENE PLAYS A ROLE IN CELL DIFFERENTIATION

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Activity of wild type p53 was suggested to be associated with either appotosis or cell differentiation. Recent experiments from our laboratory have shown that constitutive expression in pre-B cell line induced cell differentiation. Further more structural analysis have indicated that p53 promoter contains a well-conserved βHLH motif which points to the conclusion that expression of this gene is regulated by proteins of the HLH-containing family shown to be involved in a number of well-defined cell differentiation processes.

The fact that suppressor p53 functions at low molar concentrations, makes it complicated to perform a direct search for modulations in the activity of p53 protein in normal cells. Furthermore, in most systems induction of p53 expression usually causes a dramatic cessation of growth, precluding any further analysis of its function in growing cells. For these reasons, we decided to adopt an experimental model in which promoter p53 sequences in amplified copy number regulate the expression of a reporter CAT gene in transgeneic mice. Our results show that p53 expression is predominantly restricted to organs containing high frequencies of differentiating population. This *in vivo* model supports the conclusion that p53 plays a role in cell differentiation.

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THE FUNCTION OF THE P21RAS ONCOPROTEIN.

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p21ras used to be the most prominent oncoprotein in human tumors. Recent results provide a framework for the function of this protein in the control of cell growth and differentiation. p21ras mediates signals generated by activated receptor tyrosine kinases, resulting in the activation of, among others, extracellular signal-regulated kinases (ERKs). The signalling pathway from receptor tyrosine kinases to ERK2 has largely been elucidated. First, the SH2-SH3 domain containing protein Grb2 binds to the receptor and thereby translocates the exchange protein mSos to the membrane. mSos activates p21ras by bringing p21ras in the GTP-bound form. This will result in the activation of a protein kinase cascade including the raf1 kinase and ERK2. In this pathway there are several points of interference. For instance, dominant negative mutants of p21ras can inhibit p21ras specifically. Other possibilities to interfere in this pathway will be discussed. (Supported by the Dutch Cancer Society)

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PTC IN THYROID TUMOURS

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The thyroid provides an attractive model to study the steps that are involved in the neoplastic process. Tumors of the follicular epithelium of the human thyroid gland represent a multi-stage model of epithelial tumorigenesis. In fact even though most thyroid neoplasms originate from a single cell type, the thyroid follicular cell, they comprise a broad spectrum of tumors with different phenotypic characteristics and variable biological and clinical behaviour.
These tumors have been investigated for genetic alterations. A new oncogene, denominated PTC was found activated in more than 20% of the human thyroid papillary carcinomas. This gene derives from the fusion of the tyrosine kinase domain of the RET proto-oncogene and the 5' terminal region of another gene named H4. Thus a coding sequence 354bp long that belongs to the H4 gene replaces the truncated transmembrane and extracellular domains of the RET proto-oncogene.
An intrachromosomal rearrangement generates the chimaeric PTC oncogene. In fact both H4 and RET genes are located on the long arm of chromosome 10q 11.2 and q21, respectively at a distance of at least 280Kb. A chromosomal inversion (10) (q11.2-q21) is responsible for their fusion. The activation of PTC is restricted to carcinomas of the papillary histotype. In fact more than one hundred thyroid tumors other than papillary resulted negative for PTC activation. The same result was obtained by analysing 600 no-thyroid tumors. Studies are in progress to assess whether PTC activation represents an early or late event in the process of thyroid carcinogenesis.

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THE MOLECULAR BASIS OF P53 FUNCTION

J. Jenkins

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MULTIFACTORIAL ANALYASIS OF P53 ALTERATIONS IN HUMAN CANCER

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p53 alterations are involved in the majority of human malignancies. Actually, three approaches can be used to test p53 condition in human tumor : molecular, immunocytochemical and serological diagnsis.

i) Molecular analysis (1)

More than1500 p53 point mutations have been described in a large panel of human malignancies. Extensive analysis of these mutations led to the demonstration that p53 gene is an informative model useful to study the molecular mechanisms of mutagenesis in the human genome. We have analyzed the biochemical and biological behavior of several mutants. Common mutation such as Arg^{175} - His^{175} has properties characteristics of mutant p53 (no transactivationm absence of inhibitionof cellular proliferation). Nevertheless, some p53 mutants retain a wild type of conformationand are able to transactivate in a manner similar to wild type p53 suggesting that mutations are not always correlatedwith an altered p53 protein.

ii) Immunocytochemical analysis (2)

p53 mutations induce a changein the conformation of the p53 protein leading to the stabilization and the accumulation of p53 in the nucleus of tumoral cell. A new panelof monoclonal antibodies to p53 have been produced and anayzed. The localization of their epitopes have been mapped using the synthetic peptides and their pattern of recognition tested with eight different p53 pieces. Some of the; can be used for immunochemical detection of p53 in tumoral tissues from various species.

iii) Serological analysis (3)

Using a highly sensitive ELISA procedure, we have tested the sera of more than 500 patients with various neoplasia for the presence of p53 antibodies (breast, lung prostate carcinomas or B cell lymphoma). p53 antibodies are found in the area of 5% to 30% of patients depending of the tumor type. In breast carcinomas, there is a good correlation between the presence of these antibodies and the bad prognosis associated with the tumor.

Taken together, all these results indicate that a multifactorial analysis of p53 alterationis now currently available. Further works to correlate all these parameters together and also to the properties of the p53 protein will allow to distinguish the various behavior in a tumor cell.

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MOLECULAR GENETICS OF HUMAN LYMPHOMAS AND LEUKEMIAS

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