Gene Activation Studied by Immunological Methods

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Gene activation can be studied at several levels: transcription (mRNA), translation (proteins), or phenotypical alterations (functional activity or morphology). These levels can be studied in situ or biochemically by the use of specific probes for normal or altered DNA, mRNA, or proteins. Immunological probes are potent tools for studies of alterations induced by xenobiotics in target organs. When the effects of xenobiotics are studied in whole tissue, the cellular heterogeneity of the organ must be taken into account. For this reason, combined in situ and biochemical techniques are necessary. Antibodies to normal or altered cellular constituents are used for identification, quantitation, and cellular localization of proteins and modified DNA. Many xenobiotics alter gene activation by interactions with DNA. After activation, 2-acetylaminoflourene (AAF) forms DNA adducts, which can be identified immunologically. Combined with bromodeoxyuridine (BrDU) pulse labeling, techniques have been developed to demonstrate reduced adduct concentrations in proliferating cells and preneoplastic foci in the livers of AAF-fed rats. Carcinogen-induced DNA modifications are implicated as a major mechanism of altered gene activation in neoplasia, leading to phenotypical alterations. Also, cellular differentiation may be affected by xenobiotics. Differentiation-associated markers can be used for studies of gene activation. In mouse skin, the keratins K1 and K10 are only expressed in suprabasal, differentiating cells. BrDU pulse chase experiments combined with double immunofluorescence have revealed that K1 and K10 are sequentially turned on 18 to 24 hr after DNA synthesis and are followed by suprabasal migration. After a single application of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), cell migration starts directly after mitosis. Keratin K1 and K10 were still turned on 18 to 24 hr after mitosis. The experiments demonstrated that TPA induced accelerated cell migration. Initiation of cell maturation after replication was not affected by TPA, while transition through the maturation program was significantly accelerated. Gene activation can also be studied with immunological probes to the proteins that regulate gene expression, such as growth factor receptors, signal mediators, and transcription factors. It must be kept in mind that immunological detection reveals the presence of proteins, not their activity. Functional activity may be related to intracellular localization. The localization of protein kinase C, c-fos, and c-myc proteins, for example, may be altered by their activation. In other cases, activation may be detected by demonstration of phosphorylation, dimerization, or DNA binding. Western blotting and immunofluorescence have demonstrated increased expression of the regulatory units of cAMP-dependent protein kinase in rat liver after partial hepatectomy (PH) and when PH is combined with dietary AAF for growth inhibition. Immunological techniques may be powerful tools for characterization of gene activation. The specificity of the reagents needs to be extensively controlled under the employed conditions. For studies in whole tissue, immunohistochemical techniques give the additional opportunity to identify gene activation or expression in subpopulations and individual cells. — Environ Health Perspect 102(Suppl 6):205–207 (1994)

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Gene Activation
Gene activation may lead to mRNA transcription followed by translation into proteins. This process is regulated at different levels: at the transcriptional level, by post-transcriptional modifications, at the translational level, or by protein degradation. Different signaling pathways regulate these mechanisms. Xenobiotics may interfere with these processes at several levels. Modifications of DNA may alter the regulation or transcription of genes or the coding sequence. Xenobiotics also may interfere with the regulatory mechanisms of the genes and thus cause altered expression. Immunological techniques can be used for detection of normal and altered cellular structures and molecules. Techniques have been established for quantitation, cellular or intracellular localization, and molecular weight characterization of antigens. This presentation will focus mainly on the use of immunohistochemical techniques for studies of gene activation and altered gene expression. It must be emphasized, though, that additional techniques like Western blotting are required for characterization of antibodies and respective antigens.

DNA Modification
Carcinogens may cause cell alterations by DNA binding, which may lead to mutations. In rat liver, 2-acetylaminoflourene (AAF) is metabolically activated and forms DNA adducts. The dominating and carcinogetic adduct is the deacetylated aminoflourene-DNA (AF-DNA) adduct. This adduct can be identified with an antiserum to N-(deoxyguanosine-8-yl)-2-aminoflourene. This antiserum has been used for characterization of AAF-DNA modifications during continuous exposure to 0.025% AAF in the diet (1). Immunohistochemistry has been used for in situ localization. Frozen liver sections were stained by double immunofluorescence to localize AF-DNA adducts by an indirect FITC-labeled staining sequence, combined with Texas Red-labeled indirect immunofluorescence for keratins in bile ducts. A predominantly periporal localization of AF-DNA adducts was revealed (2). Adduct formation can also be studied in specific subpopulations of cells. Proliferating cells were pulse labeled with the thymidine analog 5-bromodeoxyuridine (BrDU) and identified with an anti-
body to BrdU. Double immunofluorescence for AF-DNA adducts and BrdU has been used to demonstrate reduced AF-DNA adduct staining in replicating liver cells during continuous AAF exposure, compared to surrounding tissue (3). In proliferating foci, which appear after 4 weeks of AAF exposure, even lower AF-DNA adduct levels were found. Computerized image analysis was used to quantitate in situ relative AF-DNA adduct levels in different subpopulations (4). Quantitative immuno-histochemistry may be a valuable tool for studies of the initial mechanisms of DNA modifications altered gene expression during carcinogenesis.

Cellular Differentiation

Xenobiotics may alter cell differentiation either by phenotypical alterations or by changed differentiation kinetics. This may be studied with antibodies to specific differentiation products. In epidermis, the terminally differentiating keratinocytes express the keratins K1 and K10 (5). Thus, these keratins are found in suprabasal epidermis, while the proliferating basal cells are negative for these keratins (6). Filaggrin is expressed later during differentiation. This protein is located in the granular layer of epidermis and participates in the organization of keratin filaments into bundles (7). The differentiation kinetics of keratinocytes can be studied by BrdU pulse chase experiments. Skin sections from different time points following a BrdU injection were stained by double immunofluorescence for BrdU and either of the differentiation markers, keratins K1, K10, or filaggrin. In this way, the suprabasal migration and sequential expression of the BrdU-labeled cell cohort could be studied (8). In normal mouse skin, keratin K1 was expressed 18 hours after DNA synthesis, followed by K10 expression and suprabasal migration after 24 hr. After 96 hr, the cells turned on filaggrin. The effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) on epidermal differentiation were studied in a similar experiment (A. Heyden et al., submitted). Sixteen hours following topical application of TPA, BrdU was injected. Skin sections from different time points following BrdU labeling revealed that, although suprabasal migration now started immediately, expression of keratins K1 and K10 was not accelerated. Filaggrin was expressed at 30 hr after labeling. These experiments demonstrated that in TPA-induced hyperplasia the initiation of cell differentiation was not accelerated, while cellular migration and passage through the differentiation program was faster than normal.

Oncogenes and Signal Transducers

Gene expression is regulated by growth factors and other signaling molecules that bind to cellular receptors (9). Several signal transduction pathways may then be triggered, activating transcription factors that regulate mRNA transcription by binding to DNA. Alterations in the genes of many of these cellular regulators may lead to carcinogenic transformation. These genes have been termed oncogenes. Some growth factors and receptors are associated with induction of cell replication, such as epidermal growth factor receptor, while others may induce differentiation, such as c-neu (10). In a similar way, some transcription factors like c-fos and c-jun are induced during preparation for DNA replication, while others are expressed in terminally differentiated cells, such as CCAAT/enhance-binding protein (11). These proteins may be detected by immunological probes. Different strategies may be used to demonstrate activation of these pathways. Ligand binding and polymerization may be detected by immunological coprecipitation techniques. In contrast to most other enzymes, many of the signal receptors and signal transducers are activated by phosphorylation of tyrosine. Antibodies to phosphotyrosine are therefore often useful for such studies. Also, many signal transducers are translocated when activated; growth factor receptors are internalized upon ligand binding, and many transcription factors are translocated from the cytoplasm to the nucleus upon activation (12). Combined immunochemical and immunohistochemical techniques may therefore be necessary for characterization of these regul
oratory pathways. Cyclic AMP-dependent protein kinases (PKA) are responsible for signal transduction of specific protein hormones. The regulatory unit RII of PKA has been shown to be associated with cellular differentiation (Figure 1). Activation of PKA leads to DNA binding of the cAMP-responsive element binding protein (Figure 2) (13). Studies of PKA RIIα expression in the liver have demonstrated induction of this protein 18 hr after growth induction by partial hepatectomy. This induction persisted for 14 days. When partial hepatectomy was performed on rats that were continuously fed 0.02% AAF, the expression of RIIα was delayed until 48 hr post-hepatectomy and diminished after 1 week. Such modifications in the regulation of signaling pathways may participate in the growth inhibitory effects of AAF.

Conclusion

Immunohistochemical techniques can be used to study several aspects of genetic damage and gene expression: DNA modifications, cellular differentiation, and signaling pathways. Antisera and their antigens need to be extensively controlled under the employed conditions. Especially when such studies are performed in whole tissue, combined immunohistochemical and immunohistochemical techniques are necessary to elucidate effects in different cell populations. Immunohistochemical techniques give the opportunity to identify gene activation or expression in subpopulations and individual cells.

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