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The promoter region of the Ha-ras oncogene contains CG clusters similar to those found in eukaryotic house-keeping genes, whose expression has been found to be modulated by DNA methylation. We have inserted fully methylated Ha-ras oncogene in NIH-3T3 cells by co-transfection with a plasmid containing a selectable marker. The NIH-3T3 transfected cells remained normal in morphology and were not tumourigenic in vivo. A cloned NIH-3T3 cell line, containing an integrated, methylated and silenced Ha-ras gene, was treated with the demethylating analog 5'-azacytidine. After the treatment: (1)the morphology of the cells turned from flat to reflactile spindle shape; (2)the cells acquired the property to growth in 0.3% agar and to form tumours when injected in nude mice; (3)the promoter region (0.8 SacI fragment) of the Ha-ras gene was specifically demethylated; (4)the cells produced Haras associated mRNA and p21. These data show that DNA methylation can modulate the expression of a genetically altered human ras oncogene.

MECHANISM OF ACTION OF IMMUNOTOXINS

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Immunotoxins, ITs, represent a new class of pharmacological agents designed to have cell specific activity. They are conjugates of antibodies against cell surface antigens with highly active toxins, such as diphtheria toxin and the plant toxins ricin and abrin (or their active subunits), which act by inhibiting cellular protein synthesis. The mechanism of action of ITs, and their potential use in cancer treatment have been evaluated.

The ITs are internalized by receptor mediated endocytosis. The subsequent penetration of the toxic moiety, the A-chain, through the vesicular membrane into the cytosol is somehow facilitated by the toxin B-chain and seems to involve several pathways. The toxicity and specificity of ITs depends, not only on the antigen, the antibody and the toxin used, but also upon inherent metabolic properties of the cells. Thus, different melanoma cell lines differed widely in sensitivity to abrin-IT and these differences were associated with concomitant differences in sensitivity to native abrin, probably reflecting different abilities of the cell lines to translocate the toxin from vesicles to the cytosol.

ORAL CONTRACEPTIVES AND HORMONE-RELATED CANCERS

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Our understanding of endometrial cancer, the 'simplest' hormone dependent cancer, has become ever more clear over the last 2 decades. We now have a very satisfactory semi-quantatitive explanation of the major risk factors, and in particular the markedly protective effect of combination-type oral contraceptive (COC) use, in terms of hormone levels and cell cycling times. Our understanding of breast cancer has remained, however, at a 'primitive' level. Breast cancer shares many features with endometrial cancer and the biology of the 2 diseases was until quite recently thought to be similar. Breast cancer fails, however, to show a very significant increase with oestrogen replacement therapy, or a decrease with COC use. The basis of these discrepancies has been the subject of the present investigation, and specifically, possible ways in which a breast cancer protective OC may be formulated, are under evaluation.

EFFECT OF IFN ON THE EXPRESSION OF N/C-myc AND CLASS I ANTIGENS

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The MHC class I antigens play an important role in the host defence mechanisms against tumour cells. In several laboratories it has been shown that expression of these antigens is down-modulated by some oncogenes (e.g. myc or E1A) and increased by interferon.

By immunoblotting experiments we found that after gamma-IFN treatment the class I antigens are greatly enhanced in all cell lines examined. Unlike MHC class I antigens, myc proteins appear to be slightly decreased (c-myc in melanoma and COLO 320 cells) or unaffected (N-myc in neuroblastoma and retinoblastoma Y 79 cells).