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INVOLVEMENT OF p53 IN CELL FATE AND CANCER.

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The p53 gene is a major target for genetic alterations in human cancer. Most frequently, such alterations are manifested as point mutations within the protein coding region. The main purpose of these alterations is to eliminate the tumor suppressor activity of the wild type (wt) p53 protein. However, at least some of the mutant forms of p53 that accumulate in cancer cells may also possess a gain of function, which allows them to contribute actively to the malignant properties of the tumor.

Attempts to elucidate the basis for the ability of p53 to act as a tumor suppressor have mainly been based on the forced introduction of wt p53 into transformed cells. Such studies have revealed that wt p53 activation can elicit a variety of biological responses, depending on the cellular context. In many cases, the consequence was a growth arrest, often primarily in G1. Thus, wt p53 can act as a negative regulator of cell cycle progression. In addition, wt p53 can also elicit apoptosis, and may be involved in mediating the induction of apoptosis in cells deprived of survival factors. Activation of wt p53 can also stimulate differentiation, and p53 levels increase strongly in certain differentiating tissues. Finally, there is compelling evidence that p53 mediates the response to DNA damage.

At the biochemical level, many studies indicate that p53 acts as a transcriptional regulator. The wt p53 protein can bind to specific DNA sequences and activate the transcription of genes containing such p53 binding sites. In addition, excess wt p53 can also repress transcription from many promoters. The identity of the critical target genes for p53 is still under investigation.

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MODULATION OF TUMOR GROWTH, ANGIOGENESIS AND METASTATIC CAPACITY BY HEPARIN-LIKE MOLECULES

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The pluripotent, heparin-binding, angiogenic factor, basic fibroblast growth factor (bFGF) was extracted from the extracellular matrix (ECM) produced by cultured endothelial cells and was identified in epithelial and endothelial basement membranes of the rat fetus, bovine cornea and human blood vessels. Our studies demonstrate that bFGF is specifically sequestered by heparan sulfate (HS) and heparin-like molecules in the ECM and cell surfaces, as indicated by its displacement by heparin, HS, or HS-degrading enzymes, but not by unrelated GAGs or GAG degrading enzymes. Cell surface and/or soluble heparin-like molecules are obligatory for binding of bFGF and vascular endothelial growth factor (VEGF) to high affinity cell surface receptors. Binding was abolished following treatment with heparanase or heparinase and restored by the addition of heparin. Hence, removal of HS by treatment with HS-degrading enzymes may provide a means to alter the susceptibility of cells to heparin-binding angiogenic factors. We suggest that HSPG on ECM and cell surfaces can locally either restrict or promote bFGF-receptor activity and that the balance between stimulatory and inhibitory species of HSPG will determine the degree and extent of bFGF-induced cellular responses. These results emphasize the significance of HS in controlling the availability of otherwise diffusable biological effector molecules to their signal transducing receptors in a variety of biological processes. Heparanase mediated degradation of HSPG is involved in cell invasion and release of ECM-resident angiogenic factors, both critical events in tumor progression. Heparanase inhibiting molecules are therefore expected to have a significant anticancerous effect. We have characterized the importance of size, sulfation and anticoagulant activity of heparin in: **i)** release of bFGF from the subendothelial ECM; **ii)** inhibition of heparanase and tumor metastasis; and **iii)** restoration of bFGF high affinity binding to HS-deficient cells. It was found that non-anticoagulant heparin species of different size, sulfation and substituted groups can be designed to elicit specific effects such as release of bFGF, restoration of bFGF receptor binding, or inhibition of heparanase activity, resulting in modulation of neovascularization, or inhibition of tumor metastasis, respectively. We have recently identified a series of negatively charged, non sulfated aromatic compounds (e.g., aurin tricarboxylic acid, 4-hydroxyphenoxyacetic acid) that mimic many of the effects of heparin (i.e. release of HS-bound bFGF, inhibition of heparanase). These synthetic non toxic compounds were 400-600 fold more efficient than suramin in their ability to revert autocrine transformation of 3T3 fibroblasts transfected with the bFGF gene fused to a signal peptide sequence. The development of heparin-mimicking, non-toxic anionic compounds may provide a new strategy to interfere with the autonomous and anchorage independent mode of cell growth involved in autocrine cell transformation and malignancy.

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RADIOTHERAPY FOR SURGEON AND MEDICAL ONCOLOGIST

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Radiotherapy takes advantage of the characteristics of ultra-short wave-length eletromagnetic waves (X- and gamma-rays) to leave energy in the middle they cross along their path. Although this energy is randomly deposited in the cells, only ionizations produced in or near the genetic material provoke measurable biological effects at the level of dose used in radiation oncology.

It is admitted that biologial effects are mediated through damage produced in the DNA structure (single or double strand breaks, intrastrand damage, etc.). As DNA damage density increases cell survival probability decreases, according to a probabilistic distribution best desribed by the Poisson law. At low levels of dosis, precisely those involved in daily radiotherapy, the dose-effect relationship departs from the classical Poisson distribution, i.e. a given dose increment results in less damage than at high doses. This is interpreted as reflecting the important capacities of the cell to repair DNA damage. An important feature of repair is that it can, under certain circumstances, be induced by radiation damage.

Repair capacities differ widely according to the tissue involved. In particular, late reacting tissues, those in which late effects of radiation are observed (fibrosis, necrosis...), repair more or more efficiently than early reacting tissues. Fractionated radiotherapy takes advantage of this difference as it allows more repair in late reacting tissues.

Depending on the depth of the tumor and on the surrounding normal structures focalized beams of different energies are used in order to irradiate with the maximal selectivity the targets designed by the radiation oncologist.

Ionizing radiations may be (arbitrarily) classified according to their penetration capacity, usually expressed as the depth at which 50 % of the dose is delivered. This is only a few mm for contacttherapy with soft X-rays (range 50-100 kV) or with interstitial radioactive sources in brachytherapy (iridium, iodine-125, etc); about 11.5 cm for a modern telecobalt unit, 15.5 cm for 6 MV photons and 21 cm for 23 MV photons of a linear accelerator. Another energy-dependant feature is the depth at which 100 % of the dose is delivered. In external radiotherapy the maximal dose is reached a few mm or cm under the surface, achieving a favourable skin-sparing in the treatment of deep seated tumors.

Eelectron beams are also of common use, especially for the treatment of superficial tumors, whose depth does not exceed 4 to 5 cm. A keynote feature of electrons is their limited penetration, so that the dose given to the depth is negligible.

The advent of the computer-era has revolutionized radiotherapy. It is now common practice to simulate a treatment on CT-images from a patient's tumor with a computerized treatment planning system. It means that the full irradiation can be previously planned and the relevant choice be made in order to optimize dose distribution by using a combination of the different beam energies available in a given department. A good radiotherapy department does not hesitate to pass patients from a machine to another in the course of the treatment, in order to take advantage to a maximal extent from its capacities.

The contribution of informatics will probably further increase in the future when irradiation machines will become fully computer-driven. Such a technique is already in use in stereotactic radiosurgery for small brain targets (tumors or arterio-venous malformations).