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GROWTH FACTOR RECEPTORS AS TARGETS FOR DRUG DEVELOPMENT

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A variety of serum factors stimulate cellular responses by interaction with a family of cell surface receptors that possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity. While receptor tyrosine kinases (RTKs) generate signals which, under normal circumstances, induce cell division or differentiation, it is now well established that abnormalities at the level of RTK structure or expression, or within the RTK-initiated signalling pathway, are involved in oncogenesis. This initially resulted from the realization that polypeptides that are known to play important roles in the control and transmission of cellular growth and differentiation signals have been found to be identical or closely related to oncogene products. The B chain of platelet-derived growth factor is the cellular homolog of the v-*sis* oncogene of simian sarcoma virus. The receptors for epidermal growth factor, and colony stimulating factor I were found to be proto-oncogene counterparts of v-*erb*B and v-*fms*, and the HER2/*neu* oncogene is a growth factor receptor that appears to be activated by diverse mechanisms.

For cancer in humans, current evidence indicates that overexpression is the prevalent defect involved in tumor progression. In vitro, it has been shown that increased expression levels in conjunction with activation by the ligand results in efficient transformation by EGF-R and other RTKs. HER2/*neu* and EGF-R gene amplification and overexpression also appears to be a frequent characteristic of human mammary, ovarian, and lung carcinomas and correlates statistically with patient survival and tumor recurrence.

Knowledge of the structures and signalling functions of these cell surface receptors provides a unique opportunity for the design of therapeutic reagents which target these proteins, with the goal of interrupting an autocrine activation cycle, downregulating the receptor, or otherwise inhibiting its signalling activity.

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BRYOSTATIN 1 REGULATES MULTI DRUG RESISTANCE BY A PKC-INDEPENDENT MECHANISM.

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Bryostatin 1 is a natural product found in marine bryozoans. Bryostatins exhibit a remarkable affinity to PKC. Bryostatin 1 binds to the regulatory domain of several PKC-isozymes. Binding is accompanied by an activation of the enzyme. Prolonged exposure leads to PKC-depletion. Evidence for a positive correlation between PKC-activity and multi drug resistance (MDR) has been presented. Elevated activities of PKC have been found to be correlated with an increased activity of the mdr-1 encoded P-glycoprotein. We have demonstrated that the exposure to bryostatin of mdr-1 transfected HeLa-cells reverses the mdr-1-mediated resistance to adriamycin and vinblastine and causes an increased retention of rhodamine 123 in the mdr-1 transfected cells. The cells employed express PKC-isozymes alpha, delta and zeta. Exposure to bryostatin depletes alpha, reduces the levels of delta and has no effect on PKC zeta. In order to investigate whether the modulating effect of bryostatin is due to PKC-depletion, PKC was depleted by TPA prior to bryostatin addition. Although TPA depletes the same isozymes as bryostatin, the modulating potency of bryostatin was not affected by PKC-depletion. Furthermore, exposure to bryostatin for 10 minutes which results in an activation and not a depletion of PKC is equally effective as a prolonged exposure causing PKC-depletion. The data clearly indicate that the modulating affect of bryostatin is independent of PKC. The data suggest that bryostatin interacts directly with the mdr-1 encoded P-glycoprotein. This conclusion is supported by the observation that bryostatin 1 competes with azidopine for binding to the P-glycoprotein.

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Activation of the Na^+/H^+-exchanger in NIH 3T3 fibroblasts expressing the *Ha-ras* oncogene requires stimulated calcium influx and is associated with rearrangement of the actin cytoskeleton.

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Ras p^{21} oncoproteins are detected in numerous tumors and play a pivotal role in oncogenesis. Expression of the Ha-ras oncogene in NIH 3T3 fibroblasts (+ras cells) results in growth factor independent proliferation and marked alteration of cytoskeletal architecture including breakdown of actin stress fiber network. Compared to identical cells not expressing the oncogene (-ras cells), +ras cells exhibit a more alkaline intracellular pH (pH_i) and a higher cell volume (CV), both of which are important mitogenic elements. They are due to a set point shift for activation of the Na^+/H^+-exchanger. Moreover, +ras cells respond to stimuli like 0.5% FCS or bradykinin with sustained oscillations of the cell membrane potential (PD) due to stimulated Ca^{2+} entry which triggers pulsatile release of calcium from internal stores and subsequent activation of calcium-sensitive K^+-channels. 10 µmol/l bepridil or 10 µmol/l nifedipine inhibits cellular Ca^{2+}-entry, oscillations of PD, protects +ras cells against actin stress fiber depolimerization, blocks activation of the Na^+/H^+-exchanger and proliferation of +ras cells. Inhibition of the Na^+/H^+-exchanger with 10 µmol/l HOE 694 inhibits pH_i increase and proliferation but does not significantly alter cytoskeletal rearrangement. In -ras cells ionomycin (0.1 µmol/l) leads to a transient increase in Ca_i and a sustained increase in pH_i. This effect is paralleled by a transient depolimerization of actin stress fiber network which cannot be inhibited by HOE 694. -ras cells treated with 10 µmol/l LiCl respond to bradykinin (0.1 µmol/l) with sustained PD oscillations, breakdown of actin stress fiber network and an increase in CV. In untreated -ras cells bradykinin causes a single transient hyperpolarization, is without effect on cytoarchitecture and leads to a decrease in CV. The results suggest that cytoskeletal rearrangement and activation of the Na^+/H^+-exchanger following expression of the Ha-ras oncogene occur as consequence of Ca_i oscillations and are prerequisites for growth factor independent proliferation of +ras cells.

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INTERACTIONS OF TGFα AND TGFβ IN THE REGULATION OF EPITHELIAL CELL GROWTH

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If epithelial cells are grown *in vitro*, then a simple model can be created in which TGFα stimulates growth & TGFβ inhibits growth. Additional evidence shows that steroid and trophic hormones will up-regulate one and, correspondingly, down-regulate the other. *In vivo*, the situation is much more complex. Epithelial cells are under the control of both blood-borne and stromal cell secreted factors. TGFβ stimulates both stromal cell function and angiogenesis, as does TGFα. Thus the balance between the two growth factors becomes less clear. This is important when assessing the invasive potential of breast, ovarian and prostate cancer. TGFβ may mediate some of the tumour stasis effects of tamoxifen but it is also found at high concentrations in relapsed breast cancer. TGFβ can be both stimulatory and inhibitory to ovarian cancer cells, depending on the dose. Neutralising antibodies have confirmed that these are direct effects of TGFβ. TGFα reversal of the effects of TGFβ can be prevented by blocking the tyrosine kinase activity of the EGF receptor. Future therapeutic avenues must take account of the interaction of these two growth factors.

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