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Meeting Report

Growth factors: their nature and role in medicine

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About 150 participants from 16 countries gathered at the above meeting organized by the Central Institute of Molecular Biology of the Academy of Sciences of the GDR, sponsored by the Biochemische Gesellschaft der DDR and Shimadzu Corporation (Europe) and held under the patronage of UNESCO. UNESCO support enabled the attendance of several participants from developing countries.

One major objective of the meeting was to discuss growth inhibitory factors in conjunction with growth stimulating factors.

Reviews of the current knowledge on the multifunctional transforming growth factor (TGF-beta) (Bascom, Nashville Thorgeirsson, Bethesda; Stoika, Lvov) clearly indicated the relevance of its growth inhibitory activity observed in vitro for several physiological and pathological situations in vivo. In liver, the growth inhibitory action of TGF-beta 1 seems to be linked to the induction of differentiation. Normal rat liver cells, transformed by a variety of methods lose their responsiveness to TGF-beta, suggesting that such a lost growth control could be involved in tumorigenesis (Thorgeirsson, Bethesda).

Interferons, another group of polypeptide growth inhibitors,(reviewed by Inglot, Warsaw) exert their growth inhibitory action, at least in some cell systems, by mechanisms distant from the antiviral response (Clemens et al., London).

Inhibitors of hematopoetic stem cell proliferation as the 'hemoregulatory peptide' (a synthetic pentapeptide reviewed by Laerum, Bergen) attract special medical interest because of a potential bone marrow protection in anti-proliferative tumor chemotherapy. The potent growth promoting effects of disulfide-linked dimers of the same peptide suggest the possibility of another level of regulation in terms of monomer-dimer conversions which might be of relevance also in connection with other factor-controlled systems. A synergism of the dimeric 'hemoregulatory peptide' as well as of several nucleosides with CSF in stimulating bone marrow stem cell proliferation might also be of medical interest for the management of leucopenia (Langen, Berlin).

Whereas Glaeser et al. (Halle) were able to identify an 'inhibitor' of the 'cell-flattening effect' required for the initiation of DNA synthesis in lens epithelial cells as a combined action of adenine and inorganic phosphate, the identity of the 'epidermal G1 chalone' is not yet clear, although extremely active preparations (active at 1 pg/ml) are available. However, the factor is by several criteria clearly distinct from TGF-beta (Richter, Heidelberg).

'Mammary-derived growth inhibitor' (MDGI), a 14.5 kDa polypeptide with growth inhibitory activity for a variety of mammary epithelial cells (Grosse, Berlin), was shown to have inhibitory effects on β-adrenergic responses in neonatal rat heart myocytes, suggesting an involvement of this signaling pathway also in the growth inhibitory action (Wallukat, Berlin). A previously shown lipid-bindig activity of MDGI could be dissociated from its anti-β-adrenergic effect by employing synthetic MDGI peptides, which do not bind hydrophobic ligands (Boehmer, Berlin).

The enormous complexity which emerged in the last few years in the diverse effects of various factors on cell growth may not only be due to the various interactions of different growth factors and growth inhibitors but might also in part be related to a complex diversity at the level of the receptors for one and the same growth effector. The A-type platelet-derived growth factor (PDGF) receptor can bind all known isoforms of

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PDGF (i.e. AA and BB homodimers, as well as AB heterodimers) whereas the B-type PDGF receptor can bind only BB homodimers with high affinity (Heldin, Uppsala). Since only BB homodimers can elicit actin reorganization and membrane ruffling in target cells, a differential signaling of both receptor types has to be assumed. Functional receptors most likely consist of dimers formed by association of either homologous or heterologous receptor monomers under the influence of the ligand. The shift of the receptor dimerization equilibrium upon ligand binding can then lead to an antagonistic action of one ligand with respect to the action of another one. This was demonstrated for the actin reorganization response elicited by PDGF-BB. In cells pretreated with PDGF-AA to down-regulate the A-type receptor, PDGF-AB behaves as an antagonist for PDGF-BB. The explanation is that PDGF-AB will occupy B-type receptors without the formation of functional receptor dimers due to the absence of A-type receptors.

In the case of the TGF-beta family, it will be interesting to see whether greater diversity at the receptor level as currently known can explain the strikingly different effects of the TGF isoforms 1–3 on the level of the different TGF-beta mRNAs (Bascom, Nashville). At the moment, the three TGF-beta species available as purified proteins seem to be largely cross-reactive with respect to interactions with the same set of three different membrane receptors, identified on the basis of affinity cross-linking.

The important field of the relevance of growth factors for the cardiovascular system was addressed by only a few contributors. The data of Sharma (Bad Nauheim) on increased expression of TGF-beta and beta-ECGF (endothelial cell growth factor, a member of the fibroblast growth factor (FGF) family) in an experimental ischemia model, clearly indicating the relevance of these factors in neovascularization in the heart, were quite interesting.

Comparatively, many contributions were devoted to the problem of the involvement of specific growth factors or their receptors in malignant proliferation of transformed cells and the possible usefulness of respective data in the diagnosis and treatment of tumor patients.

Truncations of growth factor receptors of the protein tyrosine kinase type are involved in the subversion of normal receptor function by various oncogenes. Respective receptor defects can be mimicked with suitable genetic constructs derived from the wild-type epidermal growth factor (EGF) receptor c-DNA and transfected into recipient cells (Panayotou, London). In the presence of EGF, overexpression of the wild-type receptor also results in a transformed phenotype.

A high expression of TGF-alpha has been linked with the transformed phenotype in NOG8 mouse mammary epithelial cells transformed by either the *ras* oncogene (under a dexamethasone-inducible promotor) or *neu* (Salomon, Bethesda). However, TGF-alpha seems to be a relatively poor mitogen, at least for MCF7 human mammary carcinoma cells (Van Zoelen, Nymegen). This led to the suggestion that at least some of the growth factors expressed in mammary tumors (i.e. TGF-alpha and PDGF) might affect the surrounding stroma rather than acting in an autocrine manner (Van Zoelen).

How these in vitro data relate to the in vivo situation is not clear. The EGF receptor status of human mammary carcinomas is inversely correlated with the estrogen receptor status (Smith, London; Perez, Havana) and the expression of the EGF receptor has been demonstrated as being of prognostic significance for the outcome of the disease (Perez). Of similar value is the estimation of the expression of c-erbB2 coding for a growth factor receptor with unknown ligand (the human variant of the rat *neu* oncogene) (Hynes, Basel). With data for both growth factor receptors, estrogen receptor (ER) data can be stratified to identify patient groups (ER+ and EGF-R− or ER+ and c-erbB2− with clearly better prognosis. Whereas the receptor coded by c-erbB2 can obviously undergo cross-activation by the EGF receptor (another example of receptor heterodimer formation?) (Hynes), TGF-alpha is currently believed to be the physiologically relevant ligand for the EGF receptor either as a soluble molecule or (as speculated by Bauknecht, Freiburg) in the form of its membrane located precursor, demonstrated to act on neighboring cells in vitro (Petrides, Munich). Or are high-molecular-mass forms of EGF of relevance for stimulating mammary tumor growth? K. Eckert (Berlin) identified a 43 kDa EGF-like protein in the urine of breast cancer patients and showed its activity to be correlated with typical prognostic breast cancer markers. Further data are required however, to establish the relevance of an 'autocrine loop' used by the mammary carcinoma cells to escape normal growth control.

A correlation of the expression of TGF-alpha and the EGF receptor could be found in human renal carcinoma (Petrides, Munich) and several gynecological tumors (Bauknecht, Freiburg). A better response in chemotherapy of EGF receptor-positive ovarian carcinomas, however, did not result in improved overall survival rates in these patients (Bauknecht).

Basic FGF might be linked to human brain tumors, in particular glioblastomas, where high levels of expression could be found in 11 out of 12 cases (Paulus, Vienna). Tissue necrosis was discussed as a possible mechanism for this growth factor which is normally not secreted to become released. Whether cell damage is a general way for a number of growth regulators lacking precursors with hydrophobic signal sequences (also PD-ECGF, Heldin, Uppsala and MDGI, Grosse, Berlin) to reach surface receptors on other cells or

whether the 'physiological ligands' of the corresponding receptors need yet to be discovered, is not known.

Compared to the established hormonal therapy of human growth deficiencies with growth hormone (HGH) under monitoring of the principal mediator insulin-like growth factor-I (IGFI) (Hesse, Berlin) or wound healing experiments with various growth factors, for instance TGF-beta (Bascom, Nashville), attemps to use growth factors for cancer treatment are still relatively rare. Page (Sainte-Foy, Quebec) used transferrin with success as a vehicle to target daunorubicidin to experimental intraperitoneal tumors. The report of Perez et al. (Havanna) on a clinical trial with 16 skin cancer patients treated with high doses of human recombinant EGF applied in an ointment was quite interesting. A striking remission in a number of cases was interpreted as a growth inhibition of EGF receptor-rich cells, however, a wound healing effect could not be ruled out.

Molecular mechanisms involved in metastasis might be even more promising targets for tumor therapy in the future. In addition to cell-adhesion molecules which seem to become lost in transformed cells, the recently discovered 'scatter' factors might present positive signals for spreading cells in the body (Birchmeier, Essen).

Regulation of normal growth and differentiation in the mammary gland was another important topic of the conference. An elegant model to study some aspects of mammary gland differentiation in monolayer culture use HC11 mouse mammary epithelial cells (Hynes, Basel). Only cells grown in the presence of EGF respond afterwards to lactogenic hormones with beta-casein expression. However, EGF must not be present during the lactogenic stimulation, otherwise beta-casein expression is suppressed.

MDGI is expressed in a tightly differentiation-coupled fashion (Grosse, Kurtz, Berlin) in the bovine mammary gland as demonstrated by an in situ hybridization analysis. This promted investigation of the possible differentiation-inducing effects of MDGI-derived synthetic peptides with promising results obtained in a long-term perfusion culture of mammary gland explants. The latter culture system (Binas, Berlin) will allow one to study growth factors under well-defined media conditions close to *in vivo* conditions, with regard to tissue complexity in the future.

Intracellular events involved in signaling of the growth factor action were addressed by a final group of contributors. Initiation factors seem to be the molecular target in protein biosynthesis, where intracellular Ca^{2+} levels can exert a regulatory function (Panniers, Rochester). Phosphotyrosine phosphatases in close proximity to growth factor receptor tyrosine kinases might present an effective means of counter-regulation for growth factor signaling (Panayotou, London). Recycling of EGF-EGF receptor complexes (up to 50% of the receptor in A431 cells) and the existence of functional (in terms of tyrosine kinase activity) internalized EGF-EGF receptor complexes were demontrated by Nikolsky et al. (Leningrad). Are the latter of relevance for the growth promoting effect?

Many genes have been hitherto put in a sequence at least with respect to their time of expression around the cell cycle, among them numerous cellular homologs of viral oncogenes. A number of new genes or proteins found to be correlated with a growth response, i.e. an extremely cycloheximide-sensitive (cell cycle restricting?) protein and a series of genes turned on in late G1 (Pardee, Boston) and proteins selectively expressed in exponential growth or the stationary phase of Ehrlich ascites tumor cells (p23 and p25, respectively, Bielka et al., Berlin) need further investigation to elucidate their place in the puzzle of intracellular events of growth factor signaling. A specific quenching of these genes by antisense nucleic acids as demonstrated for *jun* (Uckert et al., Berlin) or c-*fos* (Vlassov et al., Novosibirsk, Moscov, Berlin) might be the most straight-forward approach for that.