List of original publications

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On Dynamics and Gene Expression of Growth Factor Receptors in Human Cultured Skin Cells, Mainly after UVB Irradiation.

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Cells in a tissue carry out genetically based programmes for proliferation and differentiation upon induction by signals received from other cells or tissues. Most of these environmental signals are growth factors that are polypeptides. Growth factors represent a network of intercellular signalling molecules responsible for the development of a tissue or an individual and for the maintenance of integrity. The signals are received by receptors, which trigger a cascade of activities in the cytoplasm that will modulate the activity of nuclear transcription factors, thus regulating gene expression.

Communication between cells involves interactions of a signalling molecule with a receptor at the surface of the cell. Typically the receptor is embedded in the membrane and it is hypothesized that the binding of the signalling molecule causes a change in the state of aggregation of the receptor which, in turn, initiates a biochemical signal within the cell.

Growth factor receptors provide an essential link between diffusible growth factors and their intracellular

targets. Current models of signalling at cell surfaces raise important issues concerning growth factor receptor motion and associative properties of the receptors. Their lateral redistribution in the membrane, and side-to-side associations among them, are considered to be fundamental events in cell signalling.

Growth factor receptors are often found to be over-expressed in human tumours, i.e. to exist at increased concentration within the membrane, such that tumour growth may be driven by stimulatory signalling arising from excessive side-to-side receptor contacts.

Epidermal growth factor receptors (EGF-R) and platelet-derived growth factor receptors (PDGF-R) were studied since they affect cell proliferation and are important in tumourigenesis. The EGF-R plays a central role in numerous aspects of skin biology. In normal epidermis, EGF-R are located primarily on the surface of the proliferating basal cells with some receptors detected in the immediate suprabasal level. The EGF-R is important for autocrine growth of this renewing tissue, suppression of terminal differentiation, promotion of cell survival, and regulation of cell migration during epidermal morphogenesis and wound healing. In wounded skin, the EGF-R is transiently up-regulated and is an important contributor to the proliferative and migratory aspects of wound epithelialization.

Over-expression of the EGF-R is common in tumours of keratinocytic origin, particularly squamous cell carcinoma and in breast, bladder, cervix, kidney, ovarian tumours and lung cancer. EGF-R have been used for a number of years as a prognostic marker in clinical use, as over-expression is frequently correlated to a poor prognosis.

PDGF-R expression on cells is not constant, and the level of receptors at the cell surface can be modulated by external signals, for instance the expression increases during inflammation. Skin is a particular target for UV radiation (UVR)-induced damage and UVR plays a major role in the development of human skin cancers. UVB radiation is responsible for most of the carcinogenic effects of sun exposure. UVR is capable of modulating the expression and function of cytokine and growth factor receptors and adhesion molecules. UVB has been shown to act as a ligand to EGF-R and to rapidly, within seconds, induce activation of the receptor without the presence of the growth factor itself. EGF-R plays a major role in the UVR-induced responses.

In order to clarify and understand how UVR affects normal human skin cells even at single, physiologic doses, we focused on the reactions of growth factor receptors important for cell survival. We studied human skin cells, i.e. fibroblasts, keratinocytes and melanocytes, and their growth factor receptor expression on the surface of cells, reactions in the plane of the cell membrane, intracellular trafficking, and gene expression after UVB irradiation, exposure to their ligand, serum, and calcium depletion. We used fluorescence recovery after photobleaching (FRAP) to assess receptor characteristics in cell membranes, confocal laser scanning microscopy to visualize receptor internalization, ratio imaging for calcium studies, Northern blot for detection of the gene for the epidermal growth factor receptor and flow cytometry for cell surface receptor determination, and viability.

We have demonstrated that the three skin cell types display different basal EGF-R mobility characteristics in the plane of the cell membrane. UVB irradiation of the three cell types increased EGF-R mobility characteristics in all cell types, thus acting as an activator for the growth factor receptors. Addition of antioxidant enzymes; catalase and superoxide dismutase, prior to UVB irradiated cells, abolished the UV-induced receptor mobility changes, thus acting as protectors for the activation of growth factor receptors and the signals they transduce.

We have also shown that a single physiologic dose of UVB radiation alters the intracellular EGF-R distribution and intracellular transport in melanocytes. It also significantly alters the melanocyte phenotype. We were able to detect a constitutive EGF-R gene expression and showed that UVB radiation induces a time-dependent induction in EGF-R mRNA in melanocytes. Human melanocytes express EGF-R on their cell surface and UVR induces time-dependent changes in the number of receptors but the number of receptors does not correlate with the level of UV-induced

EGF-R gene expression.

Results showed that the addition of PDGF increased receptor mobility in normal fibroblasts, while the "starvation" of cells also increased their receptor mobility. We could show that changes in both intra- and extracellular free Ca2+ influence the mobility characteristics of PDGF receptors, thus altering the intracellular signalling.

The results show that UVR, growth factors and calcium, ubiquitous constituents of everyday life, all having tremendous effects in vivo, also affect human cells in vitro in studied parameters that are of importance for proliferation, survival and tumourigenesis.

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