ANALYSIS OF THE SUBCELLULAR LOCALISATION OF THE RECEPTOR TYROSINE KINASE HER-3

Summary

Receptors tyrosine kinases play a pivotal role in tumour development and progression, and its altered expression has been observed in breast cancer, ovarian cancer and various other malignant tumour diseases. For example, approximately 20-30 % of breast cancer patients show an overexpression of the human epidermal growth factor receptor 2, HER-2.

Therefore to counterpart this overexpression of HER-2, a therapeutic monoclonal humanized antibody (Herceptin®) directed against the extracellular domain of HER-2 was developed and is currently approved for clinical therapy.

Receptors are normally localized to the cell membrane where they are activated by ligand-binding to trigger an intracellular signalling network. In the last decade several nuclear receptors were identified, but the function of many of these receptors remains unclear.
To determine the subcellular distribution of the HER-2 and HER-3 an immunhistochemical analysis was conducted in ovarian and breast cancer tissues. In ovarian cancer HER-3 appear to have prognostic relevance in the patient's survival. Patients with high HER-3 expression have a poorer prognosis than patients with low HER-3 expression. Additionally, breast cancer patients have a significant association between HER-2 expression and overall survival.
Interestingly, 43.2 % of breast cancer patients showed a nuclear distribution of HER-3 with little or no HER-3 in cytoplasm. Reverse is also true where patients with cytoplasmic HER-3 had little or none localized to the nucleus. Thus, there appears to be "make-or-break" situation in the tumour samples but neither the mechanism nor the driving force behind the phenomenon is currently known. A further noteworthy observation is that cultured tumour cells have noticeably lower nuclear HER-3 expression (3-9%) compared to the patients' specimens, providing a potential explanation as to why this observation has been neglected in the past.
To investigate this phenomenon in more detail, cultured tumour cells were injected subcutaneously in nude mice and the nuclear HER-3 expression was analysed. Immunohistochemical analysis revealed high expression of HER-3 in the newly formed

tumour tissue leading to the assumption that the nuclear HER-3 expression might be associated with the three dimensional feature of solid tumours.

A main characteristic of solid tumours is the altered morphology which causes an undersupply of nutrients, oxygen and energy within the tumour. To determine whether challenging cells (with nutrient depletion or drug treatment) altered HER-3 localization, different cell lines were depleted of ATP, glucose or treated with the drug cisplatin and the expression of HER-3 analysed. ATP depletion was carried out using Oligomycin B and 2 deoxyglucose, resulting in an accumulation of HER-3 in the nucleus to all cells examined. In contrast, the response to glucose depletion was cell line-dependent. Under glucose depletion the cervical cancer cell line HeLa showed an elevated nuclear HER-3 expression, whereas the breast cancer cell line MCF-7 did not show increased nuclear HER-3 localization.

Dittmann et al. showed translocation of human epidermal growth factor (EGFR) into the nucleus after cisplatin exposure. Based on this observation, HER-3 localization was also analyzed after cisplatin exposure in MCF-7 cells. The result showed a clear nuclear translocation of HER-3. Although this effect was not comparable to the depletion of ATP and glucose, this finding provided further evidence that subcellular distribution of HER-3 is influenced by genotoxic stress.

An additional factor which was found to influence the cellular distribution of HER-3 was confluency. A subconfluent growing cell culture shows higher amount of nuclear HER-3 compared to cell culture with a cell density of 100 %. Therefore an association between proliferation and the nuclear HER-3 expression may exist.

Two main factors are linked to the signalling of HER-3: the dimerization partner of HER-3 (HER-2) and the ligand ($HRG_{\beta 1}$). Both factors were investigated to determine if they influence the subcellular distribution of HER-3. A Doxycycline-inducible HER-2 overexpressing cell line showed no increase in nuclear HER-3 expression with increased expression of HER-2. Furthermore, stimulation with $HRG_{\beta 1}$ did not have any effect on the nuclear HER-3 expression.

Overall, the present results suggest that the translocation of HER-3 to the nucleus may be an important step in its signalling process. Depleting cells of ATP or glucose, or treating with a genotoxic chemical such as cisplatin, resulted in nuclear HER-3 expression. The current study is also the first to show that HER-3 nuclear location in tumour samples are more abundant compared to cell lines, tools commonly used to study receptor signalling. Although the functional role of nuclear HER-3 remains unclear, this work provides some key initial factors that can be used to further elucidate its function. Additionally, the significant increase in

nuclear HER-3 expression in solid tumours may be in some way linked to resistance of such tumours to irradiation and chemotherapy.