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Heregulin signalling through HER-2 leads to increased epithelial cell growth

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Keywords

Breast cancer, epithelial cells, growth factor, HER-2, NDF, heregulin

Introduction

HER-2/*neu* is a member of the type I receptor tyrosine kinase family (RTK 1) of epithelial growth factor receptors. HER-2 overexpression resulting from gene amplification occurs in 25-30% of human breast and ovarian cancers and is associated with poor prognosis. Transfection and overexpression of this gene in mammalian cells confers growth advantage and induces transformation *in vitro*. The isolation of heregulin(HRG) and *neu* differentiation factor (NDF), specific activators of HER-2, has allowed the study of the biological effects transmitted through this receptor.

Aims

To evaluate the effect of HRG on human breast and ovarian epithelial cells expressing defined levels of the HER-2/*neu* receptor.

Comments

The authors have carried out a thorough and detailed study of the effects of HRG on human breast and ovarian cell line growth. They take into account cell line variability and are careful to avoid additional effects from factors present in serum. However, their use of HMECs is unfortunate since these are myoepithelial cells and therefore produce growth factors specific to this population, whose autocrine effects allow the almost self-sufficient growth of these cells in culture. This would also explain the lack of effect of HRG on these cells and the high levels of HER-1.

Methods

Levels of the RTK I family of receptors (HER-1,2,3,4) were measured by ELISA in a panel of breast (HBL100, MCF-7, MDA-MB-231, MDA-MB-435, SK-BR-3) and ovarian (CaOV3, 2008, C13) cell lines as well as normal human mammary epithelial cells (HMEC), before and after retroviral infection with a full-length human HER-2 cDNA. The behaviour of these cells was tested in proliferation, anchorage-dependent and independent clonogenic assays in 1% foetal bovine serum (FBS), with and without HRG at 1 and 10nM. The effect of HRG on tumours formed by parental or HER-2 overexpressing cells in nude mice, with or without oestrogen, was also measured. HRG production by the cell lines was assessed using quantative RT-PCR.

Results

HER-2/*neu*transfection resulted in a 10 to 100 fold increase in HER-2 receptor numbers per cell. This did not exceed the level of the SK-BR-3 line which naturally overexpresses this receptor. Concomitantly, HER-1 expression decreased and HER-3 expression increased in three of five breast cell lines.

Three distinct growth assays showed a variation in response of non-transfected breast and ovarian cell lines to HRG. All HER-2 overexpressing cells, apart from the normal HMECs, exhibited consistent dose-related growth/survival responses to HRG. Statistical analysis demonstrated a direct correlation between increased proliferation, anchorage-dependent and independent growth with the number of HER-2/*neu*receptors present.

HRG treatment increased the size of tumours formed from HBL100, MCF-7 and CaOV-3 cells in nude mice in the presence of oestrogen. The injection of HER-2 overexpressing cells resulted in even larger tumours (2-10 fold) and the addition of HRG increased their size even further (1.5-2 fold). In ovariectomised mice, both HRG treatment and HER-2 transfection significantly increased the size of MCF-7 cell tumours, thus demonstrating oestrogen independence of transfected cells.

RT-PCR showed that HMECs produced relatively high amounts of HRG. This could explain their lack of response to this ligand. HBL100 and MDA-MB-231 cells produced a tenth the HRG that the HMECs produced, whereas MCF-7, MDA-MB-435 and SK-BR-3 breast cell lines produced no HRG. Two of three ovarian cell lines produced significant amounts of HRG which increased following HER-2 transfection.

Discussion

The growth effects of HRG on a panel of breast and ovarian cell lines expressing defined levels of the HER-2/*neu* receptor were measured using both *in vivo* and *in vitro* assays. This strategy was employed to try and circumvent the possibility that any observed effects might result from unique characteristics of an individual cell line or specific assay technique. Overall, results support a growth stimulatory role for

HRG in the cell lines tested. The degree of response correlated with the level of HER-2, indicating the importance of this receptor isoform in HRG signalling.

References

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