PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

ErbB-4 dependent proliferation of ER+ breast cells

ArticleInfo		
ArticleID	:	3650
ArticleDOI	:	10.1186/bcr-1999-66628
ArticleCitationID	:	66628
ArticleSequenceNumber	:	70
ArticleCategory	:	Paper Report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 1999–12–9 OnlineDate : 1999–12–9
ArticleCopyright	:	Current Science Ltd1999
ArticleGrants	:	
ArticleContext	:	1305811

Mike Davies

Keywords

ErbB, estrogen, proliferation, ribozyme

Introduction

ErbB-4 is a member of the class I receptor tyrosine kinase family, the biological significance of which is poorly understood. Other members of this receptor family, including EGFR and ErbB-2, have been implicated in breast cancer. A wide range of agonists have been identified for different family members and both homodimerisation and heterodimersiation are important in signal transduction from these cell surface receptors. There are two isoforms of ErB-4, both of which are expressed in normal breast tissue and in most breast cancers.

Aims

To examine the importance of ErbB-4 expression in neoplastic transformation of breast epithelia.

Comments

Increasingly, the study of cell proliferation involves the interplay of multiple receptor pathways. This paper not only indicates the importance of ErbB4 in proliferation of breast cancer cells and the complexity of interaction of ErbB family members, but also suggests that there is some relationship between ErbB-4 signalling and oestrogen receptor pathways.

Methods

Cell lines (T47D, MCF-7, MDA-MB-453 and MDA-MB-231) were transfected with plasmids transcribing active hammerhead ribozymes. These transfected cell lines were treated with a variety of agonists to stimulate autophosphorylation of ErbB family receptors. Expression of ErbB family

members was analysed by Fluorescence-activated cell sorter (FACS) analysis and the cell lines were monitored for anchorage dependent and independent growth. Tumour formation was measured *in vivo* in athymic mice.

Breast tumour samples were examined by immunocytochemistry for ErbB-4, oestrogen receptor (ER) and progesterone receptor.

Results

Hammerhead ribozymes were shown to be specific for ErbB-4 and to be biologically active in down-regulation of the receptor mRNA and protein. These constructs and vector controls were transfected into two ER+ breast cancer cell lines (T47D and MCF7) containing high ErbB-4 levels, and two ER- cell lines (MDA-MB-453 and MDA-MB-231) which had lower levels of ErbB-4 protein.

Transfection of T47D with the most active ribozyme (RZ29) did not yield any stable transfections. A less active ribozyme (Rz6) was used for further analysis. ErbB-4 was down-regulated by 30% and 80% in two pooled populations of T47D/Rz6 transfectants. Autophosphorylation of ErbB-4 was similarly reduced.In both anchorage dependent and independent growth assays reduction in ErbB-4 was accompanied by reduced colony formation in T47D and MCF7, but not in MDA-MB-453 and MDA-MB-231. In ribozyme-transfected MCF7 and T47D cell lines, tumour growth was reduced, compared to wild-type of control transfectants.

A pilot study indicated that 1 of 5 benign tumours and 30 of 50 primary breast cancers showed staining for ErbB-4. Staining was membrane and cytoplasmic, but not nuclear and was negligible in stroma. A correlation was observed between ErbB-4 and ER status staining in these cancers and in a panel of cell lines. ErbB-4 staining seems inversely correlated to EGFR.

Discussion

Ribozyme mediated ErbB-4 down-regulation in ER+ cells lines expressing abundant ErbB-4 led to a reduction in ErbB-4 protein, ErbB-4 autophosphorylation, cell proliferation and tumour formation. However, in cell lines expressing lower initial levels of ErbB-4 no reduction in growth was seen despite complete depletion of the receptor. Hence ErbB-4 plays a role in proliferation of some ER+ cell lines.Disruption of ErbB-4 signalling is also likely to effect other members of the ErbB family via heterodimeristaion; and some ligands interact with more than one ErbB receptor. These results cast some light onto the interplay of different family members, which are expressed at different levels in the cell lines studied, confirming that a delicate interplay of receptors and ligands is important in control of proliferation of breast epithelial cells.

It is interesting that ErbB-4 correlated with the ER+ phenotype in cell lines and primary breast cancers, unlike other members of the ErbB family (notably EGFR and ErbB-2) which tend to correlate with ER- status.

References

1. Tang CK, Concepcion XZ, Milan M, Gong X, Montgomery E, Lippman ME: Ribozyme-mediated down-regulation of ErbB-4 in estrogen receptor-positive breast cancer cells inhibits proliferation both *in vitro* and *in vivo*. Cancer Res . 1999, 59: 5315-5322.