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The importance of AP-1 activity in human breast cancer

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Keywords

AP-1, breast cancer, Jun, oestrogen

Introduction

Transcription factor activator protein-1 (AP-1) complexes are made up of either homodimers of the Jun nuclear phosphoprotein family (cJun, JunB, JunD) or heterodimers of the Jun and Fos families. Interaction between AP-1 and the oestrogen receptor (ER) has been shown to lead to an inhibition of ER transcription. In animal models there is evidence for a role for cJun in inducing cellular transformation; previous studies using human breast cell lines suggest that AP-1 expression and activity decreases with increased transformation.

Aims

To determine the effect of overexpression of cJun in a human breast cancer cell line.

Comments

In this study cJun/AP-1 activity correlated with decreased ER activity. However, MCF-7 cells formed rapidly growing tumours in nude mice. Could this be a pathway leading to Tamoxifen resistance in breast cancer patients?

Methods

MCF-7 cells were co-transfected with the human *c-Jun* gene and the neomycin resistance gene (neo). Three clones expressing high levels of c-Jun (MCF7Jun) were selected by western blotting and their behaviour compared with that of three neo control clones. Assays included gel retardation to analyse AP-1 DNA binding activity, northern blot analysis of target genes as well as the relative levels of the *jun*

and *fos*gene family members and transcriptional activation assays. Transfected cells were also tested in MTT growth and Boyden chamber invasion assays as well as for their ability to form tumours in nude mice. Zymography and western analysis were used to identify matrix-degrading enzyme activity. ER protein levels were determined using radioligand binding and ER transcription by measuring chloramphenicol acetyltransferase (CAT) activity.

Results

All MCF7Jun clones demonstrated high levels of AP-1 DNA binding activity compared to minimal activity of control clones. Transactivation of AP-1 regulated genes led to increased expression of vimentin mRNA. Overexpression of cJun also resulted in a decrease in the levels of *junB*, *junD* and *c-fos* mRNA and an increase in *fra-1*expression.

MCF7Jun cells were larger than control cells, grew less compactly in monolayer and had an increased ability to invade both collagen and Matrigel in Boyden chamber assays using fibroblast conditioned medium as a chemoattractant. Type IV collagenase was detected in cJun transfected cells.

Of 18 nude mice injected with MCF7Jun cells, 15/18 and 13/18 (with/without ovariectomy) rapidly developed tumours in the absence of oestrogen in comparison to none of the control group. In culture, cJun-transfected cells grew slower than control cells in complete media but, unlike control cells, their growth was not affected by the addition of oestrogen and Tamoxifen. All three MCF7Jun clones did not express ER protein or mRNA and demonstrated no oestrogen-inducible ER transcriptional activity. Comparison of MCF-7 cells transfected with a variety of deletion mutants of cJun showed that only the clone containing full length cJun was unresponsive to oestrogen.

Discussion

In this study, increased expression of cJun in MCF-7 cells resulted in a change in the relative balance of genes within the AP-1 complex, which led to increased AP-1 transcriptional activity and an upregulation of the downstream AP-1 regulated genes for vimentin and type IV collagenase. The latter presumably being responsible for the greater invasion of MCF7Jun cells through a basement membrane matrix (Matrigel). The less compact growth of transfected cells suggests a loss of tight cell contacts, such as adherens junctions, as seen in other studies.

Although cJun-transfected cells grew slightly less well than control cells in complete media, they grew twice as fast in oestrogen-depleted medium. This oestrogen and Tamoxifen independence was found to be due to a lack of expression of ER. Similarly, MCF7Jun cells produced tumours in nude mice in the absence of oestrogen.

References

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