The breast tumour kinase Brk (PTK6) is found in over two-thirds of breast cancer cell lines and tumours but is not expressed in normal mammary cells. Brk has previously been shown to play a role in regulating proliferation in breast tumour cells [1]. However, in vivo, the site of Brk expression in normal tissues is restricted to nonproliferating cells that are undergoing terminal differentiation such as those in the gut or the skin [2,3]. This led us to hypothesise that Brk expression in breast tumours could be reflective of a differentiation phenotype, especially as a previous study had shown that involucrin, a marker of terminal keratinocyte differentiation, was expressed in a subset of tumours [4]. We therefore examined involucrin expression in breast tumour cell lines and patient biopsy samples. In addition we investigated whether inducers of differentiation in keratinocytes such as prolonged culture in suspension or vitamin D3 treatment could also affect differentiation of breast tumour cells.

We found that the expression of Brk in cultured cell lines correlated with involucrin expression. In addition the change in Brk expression, as a result of culture conditions, was accompanied by a change in involucrin levels. Moreover, treatment with vitamin D3 resulted in a decrease in cell numbers in the Brk-positive cell lines relative to the control treatments. The Brk-negative cell line was unaffected by vitamin D3 treatment.

These data suggest that Brk and involucrin may be coregulated and that inducers of differentiation such as vitamin D3 could be considered potential therapeutic strategies.

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References


C35 overexpression defines subsets of human breast cancer and its immunoreceptor tyrosine-based activation motif represents a novel treatment target

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C35 is a protein overexpressed in invasive breast cancer. The C35 gene is located on chromosome 17, next to ERBB2/HER2. C35 encodes a canonical immunoreceptor tyrosine-based activation motif (ITAM) sequence. ITAM-containing proteins have key signalling roles in the hematopoietic system and in oncogenic retroviruses. The ITAM interacts with Syk kinase, which mediates downstream signalling events.

C35-overexpressing breast tumours were found to be of two subsets. In one subset, C35 is coexpressed with HER2. The second subset is found within the basal-like carcinoma group. In order to evaluate the therapeutic potential of targeting C35 ITAM/Syk signalling, we utilised 3D cell cultures. Transformed cell lines act in a manner resembling their in vivo behaviour when grown in 3D cultures, on reconstituted basement membrane. Using this method, C35-expressing cells formed enlarged structures in both an ITAM-dependent and Syk-dependent manner. Furthermore, BT474 cells coexpressing C35 and HER2 formed more normal 3D structures when treated with a combination of Herceptin and Syk inhibitors.

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Why do most c-erbB-2/HER-2-positive breast cancer patients fail to respond to Herceptin?

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Background Herceptin is active in a subset of patients over-expressing the epidermal growth factor receptor (EGFR) c-erbB-2 (HER2) but it is not possible to predict which individuals will respond. Several molecular hypotheses have been proposed for how Herceptin causes tumour regression: one is that the antibody binds to HER2 and causes it to be internalised into breast cancer cells, where it is either degraded or locates to a compartment in which it can no longer signal (or signal in the same way). The present research aims to explore possible molecular and cellular mechanisms involved in resistance of Herceptin. We are also interested in identifying whether inhibiting other pathways (such as signalling via HER3) would increase the number of patients who show a response. We have created a plasmid containing c-erbB2 fused to Yellow Fluorescent Protein (c-erbB-2:YFP) and an epidermal growth factor receptor fused to Green Fluorescent Protein (EGFR-GFP). The correct sequence was obtained for both of these and we showed that they react with specific antibodies using western blotting. We have established a system in which we can express c-erbB2:YFP with or without coexpression of the EGFR labelled (or not) with GFP and add Herceptin chemically coupled to the red fluorescent compound Alexa Fluor 568 to see if there is an effect in cell trafficking. We have made a monoclonal antibody called SGP1 that recognises the extracellular domain of HER3 receptor [1] and we would like to see whether addition of a HER3-specific monoclonal antibody to Herceptin will increase its anticancer activity. If so, SGP1 antibody