Early phosphoprotein changes induced with epratuzumab, an antibody targeting CD22 on B cells

S Lumb, N Torbett, I Vendrell, H Turner, M Page, P Hales, A Maloney, B Vanhaesebroeck, P Cutillas, A Shock

UCB Pharma, Slough, Berkshire, UK; 2Activitics Ltd, London, UK

INTRODUCTION

Epratuzumab is a monoclonal antibody that targets the B-cell-specific receptor CD22, which is currently in phase III clinical trials in patients with systemic lupus erythematosus (SLE).

Following B-cell receptor (BCR) engagement, CD22 becomes rapidly tyrosine phosphorylated in the cytoplasmic domain within immunoreceptor tyrosine-based activation and inhibition motifs (ITAM/ITIM). In turn, these sites are responsible for recruiting an orchestrated manner effect molecules such as Lyn, Syk and PLC-γ2, adapters such as SHC1 and BIK1, and phosphatases such as SHP-1 and SHP-1, that may also attenuate BCR signaling.

Although epratuzumab has been shown to inhibit the tyrosine phosphorylation of Syk and PLC-γ2, the proximal phosphorylation events following B-cell epratuzumab and subsequent activation of B cells through the BCR are not well understood.

Targeted Quantification of Cell Signalling (TIQUAS™) is a label-free mass-spectrometry-based approach developed by Activitics that enables the quantification of thousands of phosphorylation sites from both cell and tissue extracts.

The objective of this work was to construct a B-cell phosphopeptide library and then to investigate the effects of epratuzumab on early phosphorylation in epratuzumab signatures induced through the BCR.

METHODS

B-cell purification

B cells were purified from human tonsils using a modified single-step enrichment method using 2-aminooxyethyloxirane-bromide-treated sheep red blood cell rosettes.

Epratuzumab treatment

Donor (n = 8) tonsil-derived B cells were pre-incubated for 1 h with either epratuzumab or IgG, control (both at 10 μg/ml) in serum-free media, followed by stimulation with anti-CD3/CD28 for 4–24 h.

The cells were immediately harvested and lysed in 8 M urea, 20 mM HEPPS, 150 mM NaCl, pH 8.0. Lysates were then separated and frozen at –80°C until required for TIQUAS™ processing (Figure 1).

Western blotting

Prior to mass spectrometry analysis of donor samples, the cell lysates from each donor were QC checked for activation with anti phosphorylated ERK1/2 (T925/202) mAbs.

B-cell phosphopeptide library creation

LC-MS raw data were compiled from a combination of experimentally treated human B cells and primary tonsil-derived B cells.

Penalized phosphopeptide quantification was performed using this database as a reference as described in Figure 1.

Prior to processing for mass spectrometry, the cell lysates from each treatment were analyzed by Western blot for confirmatory B-cell activation markers such as CD20 (data not shown) and ERK.

Data analysis

Activitics’ B-cell phosphopeptide library consisted of 4900 individual quantifiable phosphorylation sequences distributed across 1898 distinct proteins.

For each phosphosite, a log2 fold change (FC), p-value and false discovery rate (FDR corrected q-value) were returned.

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Metcancer

Metcancer is founded upon a comprehensive knowledgebase of high-quality molecular interactions, including protein-protein, protein-DNA and metabolic reactions) derived from manual curation of the biological literature and selected third-party databases. The interaction data is organized into Pathway Maps, representing canonical, well validated knowledge and Process Networks, containing broader models of cellular processes. Such network representations are used to integrate and biologically interpret quantitative high throughput screening data.

A cut-off of p < 0.05 was applied to identify enriched Pathway Maps and Process Networks using Metacancer’s One Click analysis.

RESULTS

A large B-cell phosphopeptide library was generated using the TIQUAS™ technology and the pathways differentially modulated by epratuzumab were explored.

The BCR pathway was the most highly regulated pathway, although the fold changes observed for individual phosphorytions tended to be relatively small at the point time assessed.

Many of the phosphorylation changes modulated by epratuzumab were novel and warrant further study of potential importance. The observed increase in ERK could be hypothesized that increased ERK activity may result in increased phosphorylation at T925/202 which is contained within a putative ERK phosphorylation site, thereby resulting in increased SHP-1 phosphorytation.

The Activitics technology offers great potential to explore the intracellular signaling events controlled by epratuzumab and other B-cell therapies.

CONCLUSIONS

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References


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