Identification of surrogate markers for determining drug activity using proteomics

C.M. McClelland and W.J. Gullick
Cancer Biology Laboratory, Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, U.K.

Abstract
In a high proportion of human carcinomas overexpression of the EGFR (epidermal growth factor receptor), a receptor tyrosine kinase, represents a potential target for cancer treatment. EGFR is induced to dimerize through ligand binding which activates the tyrosine kinase activity of the receptor. This catalyses the transfer of ATP’s γ-phosphate to hydroxyl groups of tyrosine residues on the receptor, creating binding sites that recruit downstream signalling proteins. New drugs, SMTKIs (small-molecule tyrosine kinase inhibitors), have been designed to inhibit the tyrosine kinase activity of the receptor, producing an anti-tumour effect. The development of surrogate markers to determine the drug activity of these new inhibitors would be of great benefit in drug evaluation and in the subsequent management of patient disease. This review describes current treatments of cancer using tyrosine kinase inhibitors and the use of proteomic analysis to identify possible markers of activity of these new drugs.

Increased selectivity of drugs for cancer patients
The common treatment options for cancer patients include surgery, radiotherapy and chemotherapy. However, new treatments are arising in combination with these which are directed specifically to the molecular changes which distinguish cancer cells from normal cells. EGFR (epidermal growth factor receptor) and other receptors in the family are currently under intense investigation as new targets [1]. Many good reviews of the EGFR family are available if the reader is interested to learn more [2]. New drugs, SMTKIs (small-molecule tyrosine kinase inhibitors), have been designed to inhibit the tyrosine kinase activity of the receptor, producing an anti-tumour effect [3]. These have the prospect of becoming a universal inhibitor for many cancer types [4]. They are also cheap to produce and easy for pharmaceutical companies to synthesize and screen.

Tyrosine kinase inhibitors
The first small-molecule inhibitors of EGFR were of relatively low affinity and non-selective. One of the first series of designed inhibitors were the tyrphostin (tyrosine phosphorylation inhibitor) compounds [5]. Subsequently Parke-Davis reported the first potent and selective EGFR inhibitor PD153035, which did not inhibit other tyrosine kinases [6–8]. This compound was considered a breakthrough in tyrosine kinase inhibitors as it was a competitive inhibitor of ATP. Later the same company produced three more tyr-}

Key words: epidermal growth factor receptor (EGFR), proteomics, signal transduction, small-molecule tyrosine kinase inhibitor, surrogate marker, two-dimensional electrophoresis.

Abbreviations used: SMTKI, small-molecule tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung carcinoma; 2D, two-dimensional; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight.

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Figure 1 | Identification of surrogate markers

Cancer cells
Serum starve
No treatment
Collect conditioned media at 72 hours
Concentrate
add SMTKI at optimised concentration for full inhibition of tyrosine kinase activity
pH3
pH10
100KDa
14KDa
MALDI TOF mass spectrometry
Mascot search of peptide fingerprints

Proteomics

The proteome is a term describing all the proteins expressed by a genome and the study of its composition is called proteomics. This is a relatively new concept emerging from the publishing of the draft of the human genome [17] and a new journal, *Proteomics*, has released its first issue at the beginning of 2001. Twenty years ago the human protein index (HPI) was established [18] but as DNA sequencing technologies improved, the human genome project took precedence. Now that the draft of the human genome has been published there is renewed interest in the proteome and proteomics.

2D (two-dimensional) SDS/PAGE

One of the main techniques employed for this new science is the established technique of 2D PAGE, separating proteins by their size and charge. Tiselius invented the electrophoresis technique in 1937 and the separation of serum proteins with a combination of paper and starch gel electrophoresis was first performed by Smithies and Poulik in the 1950s. By 1975, a 2D PAGE system had been developed which separated proteins on the basis of charge in the first separation dimension, using isoelectric focusing, followed by size in the second separation dimension, using SDS/PAGE [19]. The 2D electrophoresis approach to proteomics was chosen after significant improvement, principally to the first dimension of this process, by the development of the immobilized pH gradient. This process has been further modified with the use of state-of-the-art equipment and reagents since the first immobilized gradients were introduced [20]. The second dimension has not changed significantly but gel tanks and buffers have improved. However as described below the major advance has been the coupling of the high resolution of 2D gels with MS to identify individual proteins.

Several methods of detection of proteins have been developed. The gels can be stained with colloidal Coomassie Blue dye or a silver stain but there are also other methods. A combination of radiolabelling with [35S]methionine and silver staining has been used to give an improved visualization, localization and identification of proteins [21]. SYPRO Ruby and SYPRO Orange from Bio-Rad are some of the new fluorescent dyes available which are compatible with MS [22].

The gels are digitized by scanning and analysed using a software package. Software available on the market includes ImageMaster® 2D and Ettan Progenesis (Amersham Biosciences), PDQuest 2-D analysis software (Bio-Rad), Melanie 4 software (Swiss Institute of Bioinformatics, SIB) and the original Phoretix software (non-linear dynamics). Proteins of interest are then identified by mass fingerprinting using matrix-assisted laser desorption/ionization–time of flight mass spectrometry.
(MALDI-TOF) MS [23,24]. The information obtained allows the identification of individual proteins using fingerprint searches against databases. Mascot from Matrix Science is particularly useful and a number of databases can be searched free of charge (http://www.matrixscience.com).

**Applications to the discovery of surrogate markers for determining drug activity**

Our own use of the technique to identify surrogate markers is described in Figure 1. This depends on the action of a drug affecting gene expression which can be detected by analysis of the proteome with or without drug treatment. Identification of proteins where expression changes, particularly secreted proteins which can be detected in the blood, will allow the development of minimally invasive procedures to assay drug activity. This should also allow rapid drug dosing and scheduling to be assessed in experimental animals and could be extended to patient samples. Most, if not all, drugs will affect gene expression in some way, permitting the identification of surrogate markers for any new compound under investigation.

**References**

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