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Article

How the Science of Personalised Medicines Will Change the Clinical Management of Patients in the Pharmacy

The prescribing and dispensing of medication is currently a one-size fits all process and by association, the average pharmacy will contain an anthology of one-size fits all packets of 28 oral dose forms, which will be in turn labelled according to a pile of one-size-fits all prescriptions detailing rigidly indicated dosage regimens. The trouble is, unlike pharmaceutical dose forms, patients are not rigidly quality controlled by the pharmaceutical industry to ensure consistency. With evidence-based medicine, there has been a standardisation of patient care to a certain extent. When it comes to medicines however, we have to consider whether the clinical trials upon which we base our evidence and therefore compile the often strict prescribing guidelines in use by the National Health Service in the UK and other health systems internationally, are inherently flawed when they too are based upon the assumption that patients are homogenous in their response to medication.

Despite the media hype which promised instant cures for cancer, heart disease and AIDS, the mapping of the human genome and the emerging science of "genomics" arguably represented nothing more than a complex and highly comprehensive DNA sequence. Accordingly, the tide of excitement very soon ebbed, leaving scientists on the shoreline to get on with unravelling its significance and applying emerging concepts to health and disease. As a result, genomics now has a rapidly growing family with which to apply functional analysis to the genome.

As a consequence, we are now firmly embedded in the "omic" revolution. One of the new "omics" that has come to prominence is proteomics, which examines the downstream consequences of our genome, our proteins. Under the proteomics umbrella comes the subgroup of phosphoproteomics and secretomics. The secretome is the diverse type and number of proteins that are secreted or released from our cells and these are the types of proteins that are usually involved in cell migration, communication and signalling. Changes in the expression level and dysfunction in these types of proteins are often found in cancer. Many cellular proteins are switched on and off by kinase enzymes, for example, the tyrosine kinases (TKs), by the addition of phosphate groups. In disease, the pattern of phosphorylation is often found to be abnormal. Studying the phosphoproteome can help us to identify and establish which TK pathways are active in which particular cancers. As well as identifying novel therapeutic targets, knowledge of the TK signalling pathways

involved in a cancer, and how these TK pathways respond to a particular drug means that a rational approach to treatment choice can be made [1].

Throughout the development of modern medicine, it has been common practice to look for specific metabolites as diagnostic or prognostic markers in blood, urine or tissue samples. Therefore, it is not unreasonable to expect that global profiling of all the metabolites found in cells and tissues might also bear fruit. As metabolites are the endpoint of cellular metabolism, then the metabolomic profiling of thousands of metabolites is now yielding new information on the biochemical activity and interconnection of particular proteins and pathways in pathological conditions [2].

In addition to gathering knowledge from the different forms proteomics and metabolomics, scientists are also using microarray analysis to glean knowledge from the patterns of mRNA that are expressed (transcriptomics). Only a selection of genes is transcribed into mRNA before translation into protein in specific cells or tissues. Even within particular cell or tissue types, the array of mRNA molecules transcribed from genes at any one time, or the so-called transcriptome, is highly dynamic. It is not surprising then, that the scientific community also has an interest in how this selective expression of the genome is regulated. Genes are switched on and off by epigenetic chemical modifications such as methylation of genomic DNA and associated histones. The study of these non-sequence alterations to the genome, or epigenomic profiling, has been made possible by the introduction of high-throughput sequencing technology. Over recent years, research has identified an abundance of aberrant methylations to DNA that have been associated and mapped to different types of cancer cells [3]. As a result, a multitude of DNA methylation-based biomarkers have been identified for most classes of human cancer.

So how is the knowledge we gain from this ongoing "omics" work going to impact on future medicine? The first generation, pharmacogenomics, aims to apply data on the influence of certain families of genes on the pharmacology and pharmacokinetics of individual patients. So far, pharmacogenomic studies have focused primarily on polymorphic variations in the hepatic cytochrome P450 (CYP) mixed function oxidases, which affect the way patients with inherited enzyme polymorphisms respond to drugs such as the anticoagulants warfarin and clopidogrel, and anticonvulsants used to treat patients with epilepsy, such as carbamazapine. There is a concerted effort to increase our understanding of the underlying mechanisms, such as the dynamics of population pharmacogenetics and the evolution of haplotypes that determine drug handling. Centres of excellence have arisen such as the University of Liverpool (UK) Wolfson Centre for Personalised Medicines, based at the Liverpool Royal Infirmary, where there has been a healthy output of studies involving cohorts of patients relating to the pharmacogenomics of the drugs mentioned above as well

as those used for the treatment of AIDS, cancer, diabetes and depression [4]. Warfarin pharmacogenetics, in particular, has been studied in depth through genome-wide association studies, where large cohort sizes of 40,000 plus patients may be used to root out novel candidate genes translatable to the clinic as near patient tests to predict clinical response. We are now fully cognisant of the impact of CYP isoform 2C9 (CYP2C9) and VKORC1 as polymorphism that may have a very real impact on patients treated with warfarin, where such polymorphisms may confer a 50% variation in warfarin dose. The therapeutic implications are immense if we are to take into account the risk of morbidity to patients with a history of cardiovascular disease or interventions, where currently, treatment with warfarin necessitates extensive monitoring and economic cost due to hospital readmissions. Armed with our knowledge of pharmacogenomics and the means to develop high throughput, non-invasive and inexpensive near-patient diagnostic tests, however, all this could change. For example, the Amplichip (Roche Diagnostics) has been developed to allow diagnosis of CYP polymorphisms to help identify the "poor metabolizer" phenotype, in effect fingerprinting patients for the pharmacokinetic differences they will exhibit in response to a wide range of hepatically metabolised drugs. Such tests will be particularly useful in patients with polypharmacy. In the future, the experience of obtaining warfarin will be very different, where the pharmacogenomic testing in the community pharmacy has been successfully piloted [5]. Taking the prescribing of warfarin as a model- currently, this drug is prescribed "as directed" and patients are routinely monitored to minimise the risk of haemorrhage or thrombosis by regular measurement of the International Normalised Ratio (INR), a measurement based on prothrombin or clotting time. Part of a pharmacist's job is to make sure that these patients, who by the nature of their condition are often co-prescribed with antihypertensives, anti-inflammatories and oral hypoglycaemics for type II diabetes, are not compromised by CYP450-dependent drug interactions. Obtaining a comprehensive pharmacogenomic fingerprint will allow pharmacists to intervene and proactively optimise dosing regimens using dosing algorithms.

The implications to the way we receive medicines are far reaching. Less frequent dose changes will lead to increased adherence, fewer medication errors and as a result, improved clinical outcomes. Individualised dosing regimens will also change the way we prepare medicines, where bespoke medicines will be prepared in a modern take on extemporaneous compounding by making use of high-tech intelligent dose forms and formulation technologies[6]. These will take more than a tile or a pestle and mortar to prepare- rather they may take inspiration from the three-dimensional printing technology currently being used in high-tech dental practices for restorative dentistry. Dose forms will be designed for individual patients, taking into account the appropriate drug dosing regimen and drug combinations suitable for some but not for

all. On site, tailored formulations might also be fabricated using this technology, where oral dose forms with variable polymer content have been used to provide controlled release to order [7, 8]. The development of intelligent dose forms is now the leading edge of the field of Pharmaceutics, where a new take on traditional controlled release formulations is the to use advances in nanotechnology to develop systems where drug release may be activated in certain tissue-specific or physiological conditions, which will overcome the problem of inter and intra-patient heterogeneity. Smart hydrogels and nanocomposites can incorporate futuristic materials such as carbon nanotubes which will change their release characteristics in response to stimuli such as temperature and pH changes [9]. Carbon nanodiamonds, octahedral particles synthesised most easily by detonating carbon-based explosives, also have a drug carrier application. These are able to self-assemble into two-dimensional films or clusters with a chemical substrate, and by implanting them at the appropriate sight within a patient, for example after surgical excision of a tumour, they are able to provide a slow release of cytotoxic drug [10]. Alternatively, targeted drug delivery might also be achieved through molecular nanotransporters, drug carrier conjugates which consist of carrier and ligand modules, allowing receptor-mediated delivery of anticancer agent to tumours: the type of receptor and their extent of overexpression being highly variable between cancer subtypes and patients [11]. Advances in nanobiotechnology will bring cell-based delivery strategies, making possible the implantation of microencapsulated cells which release biological mediators under the control of an inducible expression vector, e.g. the tetracycline-inducible tTA system. Here, the transcription or repression of a gene is controlled externally via the administration of a sliding scale dose of tetracycline. The latest developments in cell-based systems use biological feedback pathways to design and incorporate an intelligent biosensor, for example, an implant containing cells engineered to produce bacterial urate degrading enzymes, where expression is induced in response to rising serum urate levels [12].

As academics carrying out our research in schools of pharmacy, we are cognisant of the dynamic range of scientists and health professionals involved in the laborious and often highly intensive process that takes a drug from conception to patient and through the clinical care pathway. With their extensive training across the pharmaceutical sciences, our future pharmacists will be involved with rational drug design and clinical trials. Whereas biomarkers assist with diagnosis and prognostication of disease as well as the prediction of therapeutic problems such as drug resistance; they also offer additional insight into novel targets and therapeutic strategies. The established modus operandi sets a scientific boundary between biological and chemical sciences, where the initial identification and validation of a novel target using molecular and cell pathology approaches is followed by rational drug design. Developments in chemical

biology, however, are turning this approach on its head, where medicinal chemistry strategies such as chemical proteomics and chemical genetics are allowing high throughput target identification and mechanistic studies. Chemical proteomics makes use of our knowledge of deregulated metabolism and cell signalling to design a chemical conjugates of a metabolite or enzyme substrate. Using affinity chromatography and mass spectrometry, we can plot the course of the "reactive warhead", allowing us to identify novel targets and biological interactions along the way. Thus, a "chemical biomarker" may be used to identify novel endogenous biomarkers and targets for a range of diseases[13]. Chemical genetics approaches are used to engineer drug resistant models by altering receptor configuration. Comparison of the effects of chemical inhibitors on the wild type versus resistant cell lines allows us to distinguish on or off target effects; the latter in turn leading us to novel drug targets[14].

Meanwhile, back at the clinical interface, and maybe because pharmacogenomics represents such a paradigm shift in the way we make use of patient data, this area has also been seized by the health economists, who aim to justify the increased cost and ethical issues bound to resonate among the healthcare professions and within patient groups in terms of clinical outcome. An article published previously in the *Future Medicine* series highlights the importance considering both the clinical and financial implications [15]. We must also consider the educational needs of the healthcare professionals involved in the delivery of personalised medicines, where schools of pharmacy are now to routinely include cell and systems biology, tissue and bioengineering, genetic screening, "omic" sciences, nanotechnology, bio-imaging, bioinformatics and biomarker detection and validation in their core syllabus[16].

Demonstrably, pharmacogenomics has reached the collective consciousness of the healthcare professions. When considering the impact of "omic" sciences- both upon medicines discovery and development as well as our future professional role- pharmacogenomics represents the tip of the iceberg. The real issue is how we can make use of the biomarkers and biomarker signatures offered by advances in the "omic" sciences, and how we can detect, analyse and apply such biomarkers to the development of medicines and the clinical journey of patients. The National Institutes of Health defines a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition [17]. A biomarker, therefore, may be a gene, protein, microRNA, metabolite or any other intracellular or extracellular entity. Within our genes, biomarkers may be functional (exonic) or regulatory, where we are unravelling the mysteries of epigenetics, splice variants and intronic

"dark matter" in the cause of increasing our understanding of which genes make the grade to become functioning proteins. Proteins, of course, may be enzymes, receptors (and their endogenous ligands). They may also be drug-activated or drug-binding receptors. Further, proteins may be intermediates of signal transduction cascades activating proliferation, survival, inflammation, metabolism, and in full circle, gene transcription itself. Of course, proteins may themselves also be transcription factors. In the meantime, biomarkers may be detected singly, in clusters and as a multi-gene or multi-protein signature. Much of the work carried out in the "omic" sciences has been aimed at identifying and clinically validating biomarkers by statistical evaluation of how well they correlate with clinical or surrogate endpoints, such as drug response, diagnosis, prognosis and disease progression. Biomarkers may be obtained invasively through analysis of surgically excised tissue or biopsies, or relatively non-invasively, via radiological imaging or from material such as circulating cells or cellular components from bodily fluids, secretions or effusions, which may be blood, saliva, sputum, urine, tears, sweat and even exhaled breath condensate.

This is where pharmacy can contribute, as although, in the UK at least, we have a National Health Service, unfortunately, the first point of contact with that healthcare system is when illness is suspected or has taken hold. A "schism" between the practice of medicine and public health has been described by some, where medicine prioritises the biological mechanism and treatment of disease, whilst public health focuses on disease prevention and health promotion. For this reason, there is a renewed emphasis on public health, disease prevention and early detection, that is to say, a health service that keeps people healthy [18]. The "omic" sciences and applications in genetic testing and counselling services can help identify risk of illness in patients before they get sick, but unless we make use of this information, there is little to be gained other than the ethical dilemma of potential handing a patient a death sentence that many or may not come to fruition. With around 12,000 community pharmacies in the UK, however, pharmacists will be able to provide a highly accessible prevention and early detection task force. The sighting of such public health services will be made possible by the use of non-invasive, reproducible and high throughput biomarker detection techniques which will provide a targeted early warning system for diseases such as cancer, diabetes and chronic inflammatory respiratory illness. Pharmacists already provide public health services such as smoking cessation, diabetes monitoring and weight management, so that the non-invasive detection of disease biomarkers in their body fluids will allow us to use a risk-based approach, based on epidemiology and known clinical correlates, to pick up these diseases in patients at most risk. Diagnostic tests are becoming more reproducible and more rapidly carried out. At the same time, pharmacists are also trained in the use of analytical chemistry techniques used in the proteomic detection of cancer biomarkers, such as mass

spectrometry and chromatography, and are therefore able to demystify the laboratory processes involved. They also stand at the interface of science and the clinic, being trained to interpret biochemical data and translate this to changes in medication response, adverse drug reactions and the adjustment and optimisation of medication regimens. Alongside the input of pharmacists into the medicines management of newly diagnosed or chronic conditions, pharmacy-based screening models will start to appear. These might include the early detection of chronic respiratory disease or lung malignancy using near patient testing of exhaled breath condensate from smoking cessation patients [19], or the detection of biomarkers of oral or even pancreatic cancers in saliva [20].

As the standard equipment used to carry out biomarker testing, such as qPCR (quantitative polymerase chain reaction) and immune-based proteomics approaches become less expensive, more compact and more user-friendly, it is not inconceivable that the community pharmacy will incorporate a "mini-lab" alongside the traditional dispensary and consultation room. With targeted analysis and appropriate interpretation of biomarker profiles, we potentially have both a clear, multi-faceted window into a patient's medical history and their current healthcare status. With the increasing role of pharmacists in public health and medicines management, if used effectively, biomarkers will become the "Rosetta Stone" by which we decipher the optimal clinical management of our patients in health through to sickness and back again.

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