Editorial

Marion Boland and Pauline M. Rudd*

National Institute for Bioprocessing Research and Training, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

Many systems and organisational features of cells are disturbed in disease. These include the pathways that regulate the expression of some 300 proteins involved more or less directly in glycan processing. All cell surface and secreted glycoproteins must travel through the endoplasmic reticulum and the Golgi compartments of the secretory pathway where the enzymatic addition of sugars to proteins and their further processing takes place. Proteins which contain appropriate glycosylation sequons in their primary sequence have the potential to acquire N-(AsnXaaSer/Thr) or O- (Ser/Thr) linked oligosaccharides. The processing of the glycans, carried out by enzymes acting sequentially, generally gives rise to a mixture of glycosylated variants of a glycoprotein, known as glycoforms. The compositions of the glycoforms primarily reflect the levels of the enzymes, the monosaccharides and the monosaccharide nucleotide donors that are within the cell in which the protein is expressed (reviewed in [22]).

The systems which underpin glycosylation processing are extremely sensitive to alterations in cellular signalling pathways that result both from disease associated mutations and changes in the cellular milieu. For example, patients with a congenital disorder of glycosylation (CDG Type IIa) have proteins containing only mono-antennary glycans because the *Mgat2* gene that codes for GnT II is mutated and non-functional. The analysis of the serum glycome of these patients is an unambiguous diagnostic for this serious condition [5]. The expression of enzymes involved in glycan processing may also be altered in cancer compared with normal tissue [16] and furthermore, can be regulated by factors

such as cytokines that may be present in the cancer cell environs [8].

Monitoring glycosylation changes is a potentially powerful way of diagnosing and following the course of many diseases as well as giving insights into pathogenesis. One early example was the finding that lower levels of galactosylated glycans (G0) in serum IgG was diagnostic of a limited number of diseases including rheumatoid arthritis [18] and that G0 levels followed disease progression and remission. Importantly, the changes in IgG glycosylation were ascribed to alterations in the relative proportions of the glycans present on healthy control IgG, and not new sugars. These findings are consistent with a disease process that results in an expansion in the population of particular clones of B cells that secrete high levels of IgG G0 glycoforms into the serum. These results do not necessarily imply changes in glycosylation pathways in the total population of B cells per se [3]. In this edition, the same authors further highlight some specific examples of glyco-biomarkers in relation to autoimmunity and in particular rheumatoid arthritis. The importance of glycosylation in the inflammatory response is further expanded upon by Gornik and Lauc in their review

In cancer, glycosylation changes can be detected in tumour tissue and in individual proteins isolated from tumours. However, it is the cell surface proteins shed from tumours and individual proteins secreted into body fluids such as serum that offer the greatest potential source of easily accessible biomarkers. Since these proteins have all passed through the same secretory pathway, it is reasonable to suppose that the cumulative effect of the glycosylation changes will be detectable in the glycomes of whole body fluids.

Historically, many disease-related changes in protein glycosylation have been primarily derived from studies

*Corresponding author. E-mail: pauline.rudd@nibrt.ie.

involving tissue samples and cell lines. The advent of more sensitive technologies in the detection of glycan signatures has facilitated the use of patient serum and so the identification of glycan biomarkers for disease diagnosis and prognosis has been realised. A number of recent initiatives have resulted in a crop of new studies, reflected by papers which identify and test glycosylated biomarkers, particularly for cancer. Some of these advances are discussed in this issue with contributions from de Loez et al. (prostate cancer), Szajda et al. (colon cancer), Peracaula et al. (prostate and pancreatic cancer), Saldova et al. (ovarian cancer) and from Mehta and Block who discuss IgG glycosylation in liver fibrosis and cirrhosis.

Several groups have pioneered such studies, including our own, employing state of the art LC, CE, affinity chromatography, 2-D electrophoretic and MS technologies. Increases in sialylation and fucosylation are the most common changes observed in the cancer glycome and may be hallmarks of disease progression [11, 14]. Other changes include the appearance of Lewis antigens and the shortening of O-glycan chains. To detail a few, Novotny and co-workers have performed studies using MALDI-MS coupled with various statistical analyses. An increase in both core and branching-associated fucosylation accompanies disease progression in breast cancer, and is supported by data from a cohort of progressively metastatic cell lines [13]. Mono, di, tri and tetra sialylated structures were also detected in the glycan pools. In a separate study of metastatic prostate cancer, the same group generated glycomic profiles through solid phase permethylation and MALDI-MS and again identified an increase in total fucosylation as being associated with disease progression [12]. In a collaborative study using IMS-MS, which can collectively provide structural and isomeric composition of glycans, specific glycan peaks, such as S1H5N4, were identified that were shown only to cluster with liver cancer samples and enabled differentiation from sclerotic samples [9]. Changes in the pattern of glycosylation are particularly relevant to liver pathology and a potential source of relevant biomarkers, since this organ has a major influence on the levels of glycoproteins circulating in the blood.

Studies from Michael Pierce's laboratory have used a combination of transcriptional profiling, lectin affinity technologies and NSI-MS/MS to identify changes in the glycome associated with breast and ovarian cancer [1,2]. In the latter, the most significant increase was in the level of bisecting N-acetylglucosamine and the enzyme associated with its biosynthesis (MGAT3).

Tri- and tetra-antennary complex N-glycans (α 1,4 and α 1,6) were also increased in ovarian tumours. These data are in agreement with our own, where an increase in the levels of bisecting core fucosylated complex N-linked glycans was detected in ovarian cancer patient sera by LC analysis [19] and which is further expanded upon in this series.

Enzymes involved in glycosylation may also be the target of altered methylation, affecting their promoter activity. Interestingly, GnT-V, whose activity has been associated with metastatic changes both *in vivo* and *in vitro*, catalyses an increase in α (1,6)-branched tetra-antennary N-glycan structures that often contain N-acetylglucosamine extensions. Recently it was shown that the form present in cancer was sensitive to an inhibitor of DNA methylation, 5-Aza-dC (although perhaps not itself a direct target) [6]. A number of glycosyltransferases involved in the synthesis of branching and the addition of sialyl group in gastric cancer cells have also been found to contain promoters with aberrant methylation [10].

It should be considered, however, that global changes in the glycome may only reflect the progression of a disease in general. Specifically, the body may respond adversely to increasing tumour burden and cachexia. Detailed analysis of glycans and their associated proteins may therefore provide tumour specific signatures. Examples are provided by work from Peracaula and co-workers [4,21]. PSA and RNase-1 are examples of proteins whose glycan content is significantly altered in cancer patients' serum compared with that from healthy seminal fluid or serum. Specifically, normal PSA contains a higher content of sialylated N-glycans compared with that detected in cancer patient sera. In separate studies, the same group showed an increase in core fucosylation (in the main sialylated biantennary glycans) of RNase1 from pancreatic cancer sera compared with that from normal patients [4]. These studies are further reviewed in this series.

Similarly, data from Miyoshi's group using LC-MS has identified an increase in fucosylated haptoglobin associated with the sera of patients with pancreatic cancer [17]. In fact, a marked increase in the tri-antennary N-glycans containing a Lewis X-type outer arm fucose at one specific glycosylation site on haptoglobin in pancreatic cancer compared with healthy individuals is considered specific for this disease. In a separate study, investigators also showed enhanced branching and antenna fucosylation in haptoglobin from prostate cancer patient sera compared with that from benign or normal patient sera [7]. These data along with our own [19]

increasingly support the role of glycosylated variants of haptoglobin as markers of disease progression.

Certain studies involving cultured cell lines have revealed proteins that are differently glycosylated compared with those from the tumour derived material. This is exemplified in Peracaula's work [21], where PSA from cancer serum contained a decrease in α 2,3-linked sialic acid and less fucosylation compared with that from a prostate cancer cell line. Another example includes the glycosylation pattern MUC1. The form present in the serum of patients with breast cancer is significantly different in its O-glycosylation profile compared with that analysed from a range of breast cancer cell lines [20]. Although model systems give useful information, glycosylation data should be interpreted with caution.

Glycan signatures and glycosylated variants of proteins that are associated with disease may prove to be valuable enough to inform clinical decisions. However, to reach this goal, they not only need to distinguish patients with disease from healthy controls, but furthermore, distinguish patients with cancer from those with benign disease. In this regard, one needs to establish whether global changes in the glycome are disease specific or whether they reflect bystander effects. One such example is inflammation, which is an integral part of many cancers and which in this series of papers is exemplified by the nature of biomarkers identified thus far. These data add to a growing debate as to the role of the inflammatory response in the aetiology of this disease [15]. Perhaps the use of animal models or primary cultures will enable tighter control of the variability associated with serum samples from diseased patients, although these models come with their own caveats. Finally, a biomarker should be amenable to accurate and sensitive quantitation and to robust, high throughput methods of analysis. The advent of more powerful technology may help to realise this, and to enable the testing of large cohorts of patients.

References

- [1] K.L. Abbott, K. Aoki, J.M. Lim, M. Porterfield, R. Johnson, R.M. O'Regan, L. Wells, M. Tiemeyer and M. Pierce, Targeted glycoproteomic identification of biomarkers for human breast carcinoma, *J Proteome Res* **7** (2008), 1470–1480.
- [2] K.L. Abbott, A.V. Nairn, E.M. Hall, M.B. Horton, J.F. McDonald, K.W. Moremen, D.M. Dinulescu and M. Pierce, Focused glycomic analysis of the N-linked glycan biosynthetic pathway in ovarian cancer, *Proteomics* **8** (2008), 3210–3220.
- [3] A. Alavi, J.S. Axford, F.C. Hay and M.G. Jones, Tissue-specific galactosyltransferase abnormalities in an experimental model of rheumatoid arthritis, *Ann Med Interne (Paris)* **149** (1998), 251–260.
- [4] S. Barrabes, L. Pages-Pons, C.M. Radcliffe, G. Tabares, E. Fort, L. Royle, D.J. Harvey, M. Moenner, R.A. Dwek, P.M. Rudd, R. De Llorens and R. Peracaula, Glycosylation of serum ribonuclease 1 indicates a major endothelial origin and reveals an increase in core fucosylation in pancreatic cancer, *Glycobiology* **17** (2007), 388–400.
- [5] M. Butler, D. Quelhas, A.J. Critchley, H. Carchon, H.F. Hebestreit, R.G. Hibbert, L. Vilarinho, E. Teles, G. Matthijs, E. Schollen, P. Argibay, D.J. Harvey, R.A. Dwek, J. Jaeken and P.M. Rudd, Detailed glycan analysis of serum glycoproteins of patients with congenital disorders of glycosylation indicates the specific defective glycan processing step and provides an insight into pathogenesis, *Glycobiology* **13** (2003), 601–622.
- [6] A.K. Chakraborty, F. Sousa Jde, D. Chakraborty, Y. Funasaka, M. Bhattacharya, A. Chatterjee and J. Pawelek, GnT-V expression and metastatic phenotypes in macrophage-melanoma fusion hybrids is down-regulated by 5-Aza-dC: evidence for methylation sensitive, extragenic regulation of GnT-V transcription, *Gene* **374** (2006), 166–173.
- [7] T. Fujimura, Y. Shinohara, B. Tissot, P.C. Pang, M. Kuroguchi, S. Saito, Y. Arai, M. Sadilek, K. Murayama, A. Dell, S. Nishimura and S.I. Hakomori, Glycosylation status of haptoglobin in sera of patients with prostate cancer vs. benign prostate disease or normal subjects, *Int J Cancer* **122** (2008), 39–49.
- [8] K. Higai, S. Ishihara and K. Matsumoto, NFkappaB-p65 dependent transcriptional regulation of glycosyltransferases in human colon adenocarcinoma HT-29 by stimulation with tumor necrosis factor alpha, *Biol Pharm Bull* **29** (2006), 2372–2377.
- [9] D. Isailovic, R.T. Kurulugama, M.D. Plasencia, S.T. Stokes, Z. Kyselova, R. Goldman, Y. Mechref, M.V. Novotny and D.E. Clemmer, Profiling of human serum glycans associated with liver cancer and cirrhosis by IMS-MS, *J Proteome Res* **7** (2008), 1109–1117.
- [10] Y.I. Kawamura, M. Toyota, R. Kawashima, T. Hagiwara, H. Suzuki, K. Imai, Y. Shinomura, T. Tokino, R. Kannagi and T. Dohi, DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer, *Gastroenterology* **135** (2008), 142–151 e143.
- [11] C. Kirmiz, B. Li, H.J. An, B.H. Clowers, H.K. Chew, K.S. Lam, A. Ferrige, R. Alecio, A.D. Borowsky, S. Sulaimon, C.B. Lebrilla and S. Miyamoto, A serum glycomics approach to breast cancer biomarkers, *Mol Cell Proteomics* **6** (2007), 43–55.
- [12] Z. Kyselova, Y. Mechref, M.M. Al Bataineh, L.E. Dobrolecki, R.J. Hickey, J. Vinson, C.J. Sweeney and M.V. Novotny, Alterations in the serum glycome due to metastatic prostate cancer, *J Proteome Res* **6** (2007), 1822–1832.
- [13] Z. Kyselova, Y. Mechref, P. Kang, J.A. Goetz, L.E. Dobrolecki, G.W. Sledge, L. Schnaper, R.J. Hickey, L.H. Malkas and M.V. Novotny, Breast cancer diagnosis and prognosis through quantitative measurements of serum glycan profiles, *Clin Chem* **54** (2008), 1166–1175.
- [14] B. Li, H.J. An, C. Kirmiz, C.B. Lebrilla, K.S. Lam and S. Miyamoto, Glycoproteomic analyses of ovarian cancer cell lines and sera from ovarian cancer patients show distinct glycosylation changes in individual proteins, *J Proteome Res* **7** (2008), 3776–3788.
- [15] A. Mantovani, P. Allavena, A. Sica and F. Balkwill, Cancer-related inflammation, *Nature* **454** (2008), 436–444.
- [16] E. Mas, E. Pasqualini, N. Caillol, A. El Battari, C. Crotte, D. Lombardo and M.O. Sadoulet, Fucosyltransferase activities in human pancreatic tissue: comparative study between can-

- cer tissues and established tumoral cell lines, *Glycobiology* **8** (1998), 605–613.
- [17] M. Nakano, T. Nakagawa, T. Ito, T. Kitada, T. Hijioka, A. Kasahara, M. Tajiri, Y. Wada, N. Taniguchi and E. Miyoshi, Site-specific analysis of N-glycans on haptoglobin in sera of patients with pancreatic cancer: a novel approach for the development of tumor markers, *Int J Cancer* **122** (2008), 2301–2309.
- [18] R.B. Parekh, R.A. Dwek, B.J. Sutton, D.L. Fernandes, A. Leung, D. Stanworth, T.W. Rademacher, T. Mizuochi, T. Taniguchi, K. Matsuta et al., Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG, *Nature* **316** (1985), 452–457.
- [19] R. Saldova, L. Royle, C.M. Radcliffe, U.M. Abd Hamid, R. Evans, J.N. Arnold, R.E. Banks, R. Hutson, D.J. Harvey, R. Antrobus, S.M. Petrescu, R.A. Dwek and P.M. Rudd, Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG, *Glycobiology* **17** (2007), 1344–1356.
- [20] S.J. Storr, L. Royle, C.J. Chapman, U.M. Hamid, J.F. Robertson, A. Murray, R.A. Dwek and P.M. Rudd, The O-linked glycosylation of secretory/shed MUC1 from an advanced breast cancer patient's serum, *Glycobiology* **18** (2008), 456–462.
- [21] G. Tabares, C.M. Radcliffe, S. Barrabes, M. Ramirez, R.N. Aleixandre, W. Hoesel, R.A. Dwek, P.M. Rudd, R. Peracaula and R. de Llorens, Different glycan structures in prostate-specific antigen from prostate cancer sera in relation to seminal plasma PSA, *Glycobiology* **16** (2006), 132–145.
- [22] A. Yan and W.J. Lennarz, Unraveling the mechanism of protein N-glycosylation, *J Biol Chem* **280** (2005), 3121–3124.