IgG Glycome in Colorectal Cancer

Frano Vučković\(^1\), Evropi Theodoratou\(^2,3\), Kujtim Thaçi\(^1\), Maria Timofeeva\(^3\), Aleksandar Vojta\(^4\), Jerko Stambuk\(^1\), Maja Pućić-Baković\(^2\), Pauline M. Rudd\(^5\), Lovorka Đurek\(^6\), Drazen Serviš\(^2\), Aninka Wennerström\(^8\), Susan M. Farrington\(^3\), Markus Perola\(^8\), Yuriu Aulchenko\(^9\), Malcolm G. Dunlop\(^5\), Harry Campbell\(^2,3\), and Gordan Lauc\(^1,10\)

Abstract

Purpose: Alternative glycosylation has significant structural and functional consequences on IgG and consequently also on cancer immunosurveillance. Because of technological limitations, the effects of highly heritable individual variations and the differences in the dynamics of changes in IgG glycosylation on colorectal cancer were never investigated before.

Experimental Design: Using recently developed high-throughput UPLC technology for IgG glycosylation analysis, we analyzed IgG glycome composition in 760 patients with colorectal cancer and 538 matching controls. Effects of surgery were evaluated in 28 patients sampled before and three times after surgery. A predictive model was built using regularized logistic regression and evaluated using a 10-cross validation procedure. Furthermore, IgG glycome composition was analyzed in 39 plasma samples collected before initial diagnosis of colorectal cancer.

Results: We have found that colorectal cancer associates with decrease in IgG galactosylation, IgG sialylation and increase in core-fucosylation of neutral glycans with concurrent decrease of core-fucosylation of sialylated glycans. Although a model based on age and sex did not show discriminative power (AUC = 0.499), the addition of glycan variables into the model considerably increased the discriminative power of the model (AUC = 0.755). However, none of these differences were significant in the small set of samples collected before the initial diagnosis.

Conclusions: Considering the functional relevance of IgG glycosylation for both tumor immunosurveillance and clinical efficacy of therapy with mAbs, individual variation in IgG glycosylation may turn out to be important for prediction of disease course or the choice of therapy, thus warranting further, more detailed studies of IgG glycosylation in colorectal cancer.

Introduction

The majority of extracellular and secreted proteins are post-translationally modified by the addition of glycans that are important structural and functional elements in the majority of biologic processes (1). A recent comprehensive report endorsed by the U.S. National Academies concluded that "glycans are directly involved in the pathophysiology of every major disease" and that "additional knowledge from glycoscience will be needed to realize the goals of personalized medicine and to take advantage of the substantial investments in human genome and proteome research and its impact on human health" (2). Glycans seem to have a particularly important role in the immune system and interindividual variation in glycosylation may affect function of the immune system on multiple levels (3). The differences in an individual's immune repertoire and the capacity to process and present antigens are an important element of cancer immunosurveillance (4, 5). In addition to natural anticancer antibodies, therapeutic antibodies provide clinical benefit to patients with cancer and have been established as "standard of care" therapy for several highly prevalent human cancers (6). Although antigen specificity of antibodies is determined by nucleotide sequence of hypervariable regions, effector functions of antibodies are largely determined by alternative glycosylation of asparagine 297 in the Fc region of IgG (7, 8). Depending on the extent of galactosylation, sialylation, and fucosylation of its glycans, IgG will activate complement, activate antibody-dependent cellular cytotoxicity (ADCC), or even have an anti-inflammatory action (9, 10). Both ADCC and complement-dependent cytotoxicity are important for function of anticancer antibodies (6), thus variation in IgG glycosylation may influence interindividual variability in cancer immunosurveillance and/or response to therapeutic antibodies.

Our recent studies demonstrated that IgG glycosylation is very variable between individuals (11), but the functional
Effects of surgery on the IgG glycome were evaluated in 28 patients sampled before surgery, 24 hours, 48 hours, and 7 days after surgery. Samples were collected at the Clinical Department for Laboratory Diagnostics at University Hospital Dubrava. The study was registered at ClinicalTrials.gov, number NCT01244022 and was approved by the Ethics Committee of University Hospital Dubrava.

Patients who were sampled before the initial diagnosis of colorectal cancer, as well as matching controls that did not develop colorectal cancer during the same follow-up time were selected from the FINNRIISK cohort (17).

Isolation of IgG from human plasma. The IgG was isolated using protein G monolithic plates (BIA Separations) (11). Briefly, 70 to 100 μL of plasma was diluted 8 times with 1× PBS, pH 7.4, applied to the protein G plate and instantly washed with 1× PBS, pH 7.4 to remove unbound proteins. IgGs were eluted with 1 mL of 0.1 mol/L formic acid (Merck) and neutralized with 1 mol/L ammonium bicarbonate (Merck).

Glycan release. IgG samples were dried in a vacuum concentrator, dissolved in 30 μL 1.33% SDS (w/v; Invitrogen) and denatured by incubation at 65°C for 10 minutes. After incubation, samples were left to cool down to room temperature for 30 minutes. Subsequently, 10 μL of 4% Igepal CA-630 (Sigma-Aldrich) were added to the samples and incubated on a shaker for 15 minutes. After shaking, 1.25 mU of PNGase F (Pro-Zyme,) in 10 μL 5 × PBS were added and incubated overnight at 37°C for N-glycan release.

Glycan labeling. The released N-glycans were labeled with 2-aminobenzamide (2-AB). The labeling mixture was freshly prepared by dissolving 2-AB (Sigma-Aldrich) in DMSO (Sigma-Aldrich) and glacial acetic acid (Merck) mixture (70:30, v/v) and by adding 2-picoline borane (Sigma-Aldrich) to a final concentration of 19.2 mg/mL for 2AB and 44.8 mg/mL for 2-picoline borane. A volume of 25 μL of labeling mixture was added to each N-glycan sample in the 96-well plate and the plate was sealed using adhesive tape. Mixing was achieved by shaking for 10 minutes, followed by 2-hour incubation at 65°C.

HILIC-SPE. Free label and reducing agent were removed from the samples using HILIC-SPE. Cellulose-based HILIC-SPE cleanup of the Hispanic cohort samples was performed as described previously (Kristic and colleagues, 2014). The 2 × 100 μL eluates were combined and stored at −20°C until usage.

HILIC-UPLC. Fluorescently labeled N-glycans were separated by hydrophilic interaction chromatography (HILIC) on a Waters Acquity UPLC instrument (Milford) consisting of a quaternary solvent manager, sample manager, and a FLR fluorescence detector set with excitation and emission wavelengths of 250 and 428 nm, respectively. The instrument was under the control of Empower 2 software, build 2145 (Waters). Labeled N-glycans were separated on a Waters BEH Glycan chromatography column, 100 × 2.1 mm internal diameter, 1.7 μm BEH particles, with 100 mmol/L ammonium formate, pH 4.4, as solvent A and acetonitrile as solvent B. Separation method used linear gradient of 75% to 62% acetonitrile (v/v) at flow rate of 0.4 mL/minute in a 25-minute analytical run. Samples were maintained at 5°C before injection, and the separation temperature...
was 60°C. The system was calibrated using an external standard of hydrolyzed and 2-AB labeled glucose oligomers from which the retention times for the individual glycans were converted to glucose units. Data processing was performed using an automatic processing method with a traditional integration algorithm after which each chromatogram was manually corrected to maintain the same intervals of integration for all the samples. The chromatograms were all separated in the same manner into 24 peaks and the amount of glycans in each peak was expressed as percentage of total integrated area.

**Statistical analysis.** Clinical characteristics among patients and controls were compared using Wilcoxon and Fisher exact tests. To make measurements across samples comparable, normalization by total area was performed where peak area of each of 24 glycan structures was divided by total area of corresponding chromatogram. Batch correction was performed on normalized log-transformed measurements using linear mixed models (R package lme4), where technical source of variation was modeled as random effect. In addition to 24 directly measured glycan structures, 12 derived traits were calculated from the directly measured glycans. These derived traits average particular glycosylation features (galactosylation, fucosylation, sialylation) across different individual glycan structures and consequently, they are more closely related to individual enzymatic activities and underlying genetic polymorphisms.

Association analyses between disease status and glycan traits were performed using a logistic regression model with age and sex included as additional covariates. Prior to analyses, glycan variables were all transformed to standard normal distribution (mean = 0, SD = 1) by inverse transformation of ranks to Normality (R package 'GenABEL', function rmtransform). Using rank-transformed variables in the analysis makes estimated ORs of different glycans comparable as transformed glycan variables have the same standardized variance, and in that case estimated ORs always correspond to one standard deviation change in the measured glycan trait. False discovery rate was controlled using Benjamini–Hochberg procedure (18).

For prediction of colorectal cancer status, regularized logistic (elastic net) regression model was applied (R package "glmnet"). For classification, only 24 initial glycan traits were used as predictors. Prior to model training and validation, elastic net regularization parameters (α and λ) were tuned on 20% of samples (260 samples), and optimal parameters chosen by the tuning procedure (α = 0, λ = 0.1) were used in further analysis. To evaluate performance of predictive model, 10-cross validation procedure was used on remaining 80% of samples. Predictions from each validation round were merged into one validation set on which model performance was evaluated on.

**Expression datasets for colorectal carcinoma.** Expression data for colorectal carcinoma were downloaded from the Gene Expression Omnibus (GEO) database (19). We picked datasets with a comparably large number of samples (49–148 samples total) that included both colorectal cancer and the matching noncancerous tissue. The datasets are stored under the accession numbers GSE867120, GSE2151021, and GSE2451422.

**Analysis of gene expression in colorectal carcinoma.** Expression data analysis was conducted using the R language and environment for statistical computing (23), which was also used for preparation of figures. Data were retrieved with the R package “GEOQuery.” Differences in expression levels with the corresponding P values for each dataset containing cancer and noncancer samples were calculated using the package “limma.” Genes were annotated according to the package and database “org.Hs.eh.db.” Results for the 20 selected candidate genes were visualized as volcano plots constructed with the aid of the package “ggplot2." In these plots, the cut-off value for significance was set well above the level of P = 0.05 adjusted using Bonferroni correction for multiple testing. The cut-off value for the fold change was picked for each individual dataset such that only the several most changed genes are selected.

**Results**

IgG glycome composition was analyzed in 760 patients with colorectal cancer and 538 matching controls. Descriptive information on colorectal cancer patients and healthy controls are presented in Table 1. Total IgG glycome (combined Fc and Fab glycans) composition was determined by UPLC analysis of 2AB-labeled glycans as reported recently (13). A typical chromatogram showing separation of the IgG glycome into individual structures is shown in Fig. 1. Many of these structures share the same structural features (galactose, sialic acid, core-fucose), thus additional derived traits were calculated that average these features across multiple glycans (Supplementary Table S1).

Significant differences were observed in several features of the glycome (Table 2; Fig. 2), primarily reflecting decreased galactosylation (OR = 2.35; P = 2.39E-22 for G0 and OR = 0.36; P = 6.59E-29 for G2) and sialylation (OR = 0.72; P = 2.73E-05 for S total), as well as increased fucosylation of neutral IgG glycans (OR = 1.24; P = 3.57E-03 for F total) and decreased fucosylation of sialylated glycans (OR = 0.72; P = 5.85E-05 for F sialo). As many glycan structures showed strong association with colorectal cancer, we attempted to build a

---

**Table 1.** Descriptive information on colorectal cancer patient and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 760)</th>
<th>Control (n = 538)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [median(IQR)]</td>
<td>52 (48–56)</td>
<td>53 (48–56)</td>
<td>0.274</td>
</tr>
<tr>
<td></td>
<td>415 (54.6%)/545 (45.4%)</td>
<td>289 (53.7%)/248 (46.2%)</td>
<td>0.821</td>
</tr>
<tr>
<td>Smoking status</td>
<td>133/172/271/138</td>
<td>94/159/21/103</td>
<td>0.836</td>
</tr>
<tr>
<td>BMI [median(IQR)]</td>
<td>26.3 (23.4–26.8)</td>
<td>27.8 (25.8–28.6)</td>
<td>2.69E–10</td>
</tr>
<tr>
<td>Family history</td>
<td>524/997/59</td>
<td>517/6/15</td>
<td>6.50E–44</td>
</tr>
</tbody>
</table>
predictive model using regularized logistic regression. Only the 24 directly measured glycan traits were used as predictors in the model. Evaluation of model performance was conducted using a 10-cross validation procedure. Although a model based on age and sex did not show significant discriminative power (AUC = 0.499), the addition of glycan variables into the model considerably increased the discriminative power of the model (AUC = 0.755; \( P < 1 \times 10^{-16} \); Fig. 3).

Glycome composition is known to change in acute inflammation (24) and to evaluate potential effects of surgery on the IgG glycome in colorectal cancer patients, we analyzed IgG glycome composition in 28 patients (i) before surgery, (ii) 24 hours after surgery, (iii) 48 hours after surgery, and (iv) 7 days after surgery. We did not observe any consistent and statistically significant changes in the IgG glycome that were caused by the surgery (data not shown).

To determine whether the observed changes were present before the disease onset, we identified 39 individuals from the FINNRRISK cohort that were sampled before the initial diagnosis. However, when compared with matching controls, no statistically significant changes were found.

Core-fucose is added to glycans by the fucosyltransferase 8 enzyme (encoded by \( FUT8 \) gene), which has recently been reported to be functionally relevant in some cancers (25–27).

**Table 2.** IgG glycome composition in colorectal cancer patients and controls. Only the main derived traits describing glycome composition are shown. Directly measured glycan structures are available in Supplementary Table S2.

<table>
<thead>
<tr>
<th>Glycan</th>
<th>Control [median(IQR)]</th>
<th>Patient [median(IQR)]</th>
<th>OR</th>
<th>95% Confidence interval</th>
<th>( P )</th>
<th>( P_{\text{adjusted}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0 total</td>
<td>25.35 (20.67–29.24)</td>
<td>29.24 (24.62–34.74)</td>
<td>2.35</td>
<td>(1.95–2.82)</td>
<td>( 2.65E-23 )</td>
<td>( 2.39E-22 )</td>
</tr>
<tr>
<td>G1 total</td>
<td>36.62 (34.87–38.09)</td>
<td>35.94 (34.49–37.39)</td>
<td>0.64</td>
<td>(0.55–0.74)</td>
<td>( 8.09E-10 )</td>
<td>( 3.64E-09 )</td>
</tr>
<tr>
<td>G2 total</td>
<td>16.65 (14.41–19.62)</td>
<td>14.12 (11.71–16.78)</td>
<td>0.36</td>
<td>(0.30–0.44)</td>
<td>( 1.83E-30 )</td>
<td>( 6.59E-29 )</td>
</tr>
<tr>
<td>F total</td>
<td>95.27 (94.42–95.97)</td>
<td>94.39 (94.40–96.11)</td>
<td>1.11</td>
<td>(0.97–1.28)</td>
<td>( 1.27E-01 )</td>
<td>( 1.53E-01 )</td>
</tr>
<tr>
<td>F neutral</td>
<td>97.07 (96.38–97.66)</td>
<td>97.32 (96.50–97.97)</td>
<td>1.24</td>
<td>(0.08–1.43)</td>
<td>( 9.86E-03 )</td>
<td>( 3.57E-03 )</td>
</tr>
<tr>
<td>F sialo</td>
<td>88.47 (86.69–89.79)</td>
<td>87.58 (85.39–89.29)</td>
<td>0.72</td>
<td>(0.62–0.84)</td>
<td>( 2.44E-05 )</td>
<td>( 5.85E-05 )</td>
</tr>
<tr>
<td>B total</td>
<td>18.25 (16.81–20.22)</td>
<td>18.11 (16.49–20.02)</td>
<td>0.88</td>
<td>(0.76–1.01)</td>
<td>( 7.68E-02 )</td>
<td>( 9.87E-02 )</td>
</tr>
<tr>
<td>B neutral</td>
<td>17.97 (16.35–20.54)</td>
<td>17.89 (16.07–19.93)</td>
<td>0.84</td>
<td>(0.73–0.97)</td>
<td>( 1.86E-02 )</td>
<td>( 2.91E-02 )</td>
</tr>
<tr>
<td>B sialo</td>
<td>18.64 (16.48–21.33)</td>
<td>19.19 (16.99–21.77)</td>
<td>1.17</td>
<td>(0.01–1.35)</td>
<td>( 3.85E-02 )</td>
<td>( 5.13E-02 )</td>
</tr>
<tr>
<td>S total</td>
<td>20.71 (18.64–23.62)</td>
<td>19.85 (17.39–21.96)</td>
<td>0.72</td>
<td>(0.62–0.83)</td>
<td>( 1.06E-05 )</td>
<td>( 2.75E-05 )</td>
</tr>
<tr>
<td>S1 total</td>
<td>15.9 (14.07–18.07)</td>
<td>14.97 (12.94–16.74)</td>
<td>0.64</td>
<td>(0.54–0.75)</td>
<td>( 9.16E-09 )</td>
<td>( 3.66E-08 )</td>
</tr>
</tbody>
</table>

Note: False discovery rate was controlled using Benjamini-Hochberg procedure (\( P_{\text{adjusted}} \)).

Abbreviations: B, bisecting GlcNAc; F, fucose; G, galactose; S, sialic acid.
Aiming to investigate the potential molecular mechanisms underlying increased IgG core-fucosylation in colorectal cancer, we examined effects of SNP that associated with fucose-related glycan traits on both glycans and colorectal cancer. A list of SNPs that showed the strongest associations with fucosylation in our recent IgG glycome GWAS are shown in Supplementary Table S3 with effect sizes and \( P \) values for both glycans and colorectal cancer. Although SNPs that associate with fucosylation did not pass correction for multiple testing for association with colorectal cancer, it is of interest that we observed nominally significant associations with colorectal cancer risk for 96 out of 100 top glycan SNPs and that the direction of the effect was consistent for both core-fucose and colorectal cancer risk in all these SNPs.

Furthermore, we examined the expression of all genes that were previously associated with the IgG glycome (13) in colorectal cancer and matching tissues in publicly available datasets. Significantly increased expression of \( FUT8 \) was observed in all datasets (Fig. 4).

**Discussion**

This study represents the first comprehensive analysis of IgG glycosylation in colorectal cancer. By applying the recently developed method for high-throughput glyco-profiling of IgG (11) on a well-characterized large cohort of colorectal cancer patients and matching controls, we have filled this important gap in knowledge which may have relevance for understanding...
the immunosurveillance of colorectal cancer (4). More generally, given the established role of IgG glycan composition in determining IgG effector function, these data may be of importance in helping to understand determinants of success of anticancer therapy with mAbs (6). By analyzing 760 colorectal cancer patients and 538 matching controls, we have found that colorectal cancer is associated with three major alterations in the IgG glycome composition: (i) decrease in IgG galactosylation, (ii) decrease in IgG sialylation, and (iii) increase in core-fucosylation of neutral glycans with concurrent decrease of core-fucosylation of sialylated glycans.

The addition of galactose and sialic acid to IgG glycans have been shown to result in the conversion of proinflammatory activity of IgG into an anti-inflammatory activity and are believed to be essential for the immunosuppressive activity of intravenously administered immunoglobulins (28). Galactosylation and sialylation of IgG decrease significantly with the age of an individual (29). The same pattern of changes in the IgG glycome have been reported in rheumatoid arthritis (30), osteoarthritis (30), inflammatory bowel disease (14, 31), systemic lupus erythematosus (15), and some other diseases (32). In this study, we have observed consistent decreases in all structures with two terminal galactoses \( \text{A2BG2 (GP13), FA2G2 (GP14), and FA2BG2(GP15)} \) and an increase in structures without galactoses \( \text{A2 (GP2), A2B (GP3), FA2 (GP4), and FA2B (GP6)} \) (Supplementary Table S2). The decrease in galactosylation was also evident in the derived traits \( G0 \) and \( G2 \) that average galactosylation in several individual glycans \( \text{G0: OR, 2.35; P, 2.39E-22; G2: OR, 0.36; P, 6.59E-29).} \)

Several directly measured IgG glycans containing sialic acid, as well as the derived trait “S1 total” (measuring all monosialylated IgG glycans) were also decreased in colorectal cancer patients (Table 2).

As decreases in galactosylation and sialylation have also been observed in a number of other diseases (32), this pattern of glycan changes (which are consistent with a decrease in the immunosuppressive potential of IgG) is not specific for colorectal cancer.

As little is known about the mechanisms of regulation of IgG glycosylation (12), it is very difficult to speculate about potential mechanisms and causes of these differences. The heritability of IgG glycosylation is relatively high. The heritability of galactosylation has been estimated to be between 40% and 70% and of sialylation between 30% and 60% (11, 33). However, the glycome has also been reported to be dynamic and capable of change [particularly in situations of disturbed homeostasis (24)].

Figure 3.
Classification of colorectal cancer patients using IgG glycans. ROC curve illustrating the performance of regularized logistic regression model in discrimination between colorectal cancer patients and healthy controls from SOCCS retrospective study. Although models based only on age and gender did not show predictive power (dotted line), addition of glycan traits increased predictive power of model (solid line).

Figure 4.
Volcano plots showing changes in expression for the 20 candidate genes in three different datasets for colorectal carcinoma (see Materials and methods section). Names of the genes above cutoffs for the fold change and the statistical significance are indicated. One gene may correspond to multiple probe sets, each of them corresponding to a point on a graph. The log2 fold change refers to the ratio of expression in noncancerous tissue to the expression in cancer tissue, which puts the genes with higher expression in cancer to the left and those with lower expression to the right. The position on the y-axis (-log10 \( P \) value) corresponds to the statistical significance of the change, with higher significance positioned higher on the graph.
Recently, it was reported that the IgG glycome changes before the onset of rheumatoid arthritis (34, 35), indicating that they may be a part of disease pathophysiology. In an analogous attempt to address causality in this study, we were able to identify 39 individuals that were sampled before the initial diagnosis of colorectal cancer. However, when compared with matching controls (individuals of the same age that did not develop colorectal cancer in the same period after recruitment), we did not identify any statistically significant differences. As the sample size was very small, it is hard to derive any conclusions from this exploratory part of the study, beside the fact that we were not able to show any differences in IgG glycome composition before the onset of colorectal cancer.

Activation of ADCC is believed to be an important mechanism of therapeutic mAbs, as indicated by the fact that a common SNP in FcγRIIIa (V158F) is correlated with clinical responses to cetuximab (36) and rituximab (37). The attachment of fucose to the glycan core (core-fucose) is a mechanism that interferes with binding of IgG to FcγRIIIa and greatly diminishes its capacity to activate ADCC (38, 39). The removal of core-fucose from IgG glycans increases clinical efficacy of mAbs, enhancing their therapeutic effect through ADCC-mediated killing (40–42). Recently, it has been shown that properly glyco-engineered antibodies (43) can efficiently elicit ADCC even in immunocompromised colorectal cancer patients (44). This observation indicates that efficient immunosurveillance of tumor cells depends on antibody/Fc receptor affinity. Thus, increased levels of core-fucose on neutral IgG glycans in colorectal cancer patients may influence disease risk and could be used to decrease the binding ability of IgG to activate ADCC. Interestingly, core-fucose was increased in sialylated glycans. To the best of our knowledge, this is the first report of different direction of changes in fucosylation in sialylated and neutral glycans. Recent studies clearly demonstrated that some antigen-specific antibodies can have significantly different levels of fucose (45), but it is hard to evaluate the importance in the different fucosylation of sialylated and neutral IgGs, as the relevance of sialylation on the impact of fucose on IgG function is currently not known (46).

This is further supported by the observation that polymorphisms in the FUT8 gene seem to be associated with increased risk for colorectal cancer. In our relatively small dataset, this association did not remain statistically significant after correction for multiple testing. Nevertheless, we suggest that this is an interesting and potentially important observation. It is supported by our finding that 96 out of 100 top glycan SNPs were also nominally significant for colorectal cancer risk, with directions of effect that were consistent (Supplementary Table S1) with our hypothesis. A larger study would be needed to confirm this association, but if replicated, this observation would indicate that individual variation in IgG fucosylation affects risk for the development of colorectal cancer.

Additional evidence supporting a possible functional importance of FUT8 in colorectal cancer is the recent observation that microRNA MiR-198 represses tumor growth and metastasis in colorectal cancer by targeting FUT8 (47). Our analysis of transcriptional data in publicly available datasets also revealed significantly increased expression of FUT8 in colorectal cancer (Fig. 4). The finding that similar pattern of changes in FUT8 expression can be observed or inferred in both the tumor tissue and the antibody-producing B lymphocytes points to the importance of general mechanisms controlling fucosylation in colorectal cancer and implies that the same features of genetic makeup influence glyco-gene expression (and thereby glycosylation profile) in both the tumor tissue and in B cells. We recommend that attempts should be made to replicate the findings of this “hypothesis-generating” study in other datasets. In particular, the association between increased levels of core-fucose on neutral IgG glycans and colorectal cancer patients warrants further investigation given their potential therapeutic implications and translational potential.

Decreases in galactosylation and sialylation have been previously observed in a number of diseases, including in our recent large studies of inflammatory bowel disease (14) and systemic lupus erythematos (15). Thus, these glycan changes do not appear to be specific for colorectal cancer. However, the increase in core-fucose on neutral glycans, with concurrent decrease of core-fucose on sialylated glycans has not been previously reported.

In this study, we have demonstrated significant differences in IgG glycome composition between colorectal cancer patients and controls. We were not able to detect these differences in historical samples (taken before colorectal cancer had developed in these patients). This could indicate that the changes are due to reverse causality (due to the disease process or treatment effects). However, it may also be due to inadequate study power in this small study and so additional studies are required to investigate this further. Considering the functional relevance of IgG glycosylation for both tumor immunosurveillance and clinical efficacy of therapy with mAbs, individual variation in IgG glycosylation may turn out to be important for prediction of disease course or the choice of therapy, thus warranting further, more detailed studies of IgG glycosylation in colorectal cancer.

Disclosure of Potential Conflicts of Interest

Y. Aulchenko has ownership interest in PolyOmica. G. Lauc has ownership interest (including patents) in and reports receiving commercial research support from Genos. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: E. Theodoratou, P. Rudd, A. Wennerstrom, M.G. Dunlop, H. Campbell, G. Lauc


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Theodoratou, M. Pučić-Baković, L. Lyerek, D. Servis, A. Wennerstrom, S.M. Farrington, M. Perola, M.G. Dunlop, H. Campbell, G. Lauc


Writing, review, and/or revision of the manuscript: F. Vučković, E. Theodoratou, M. Timofeeva, A. Voja, M. Pučić-Baković, P. Rudd, A. Wennerstrom, S.M. Farrington, M. Perola, Y. Aulchenko, M.G. Dunlop, H. Campbell, G. Lauc

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Vučković, E. Theodoratou, P. Rudd, S.M. Farrington, M. Perola

Study supervision: H. Campbell, G. Lauc

Grant Support

This work was supported by programme grant no. C348/A12076 from Cancer Research UK, EU HighGlycans FP7 grant, E. Theodoratou has a
Chancellor’s Fellowship from the University of Edinburgh. Glycan analysis was partly supported by European Commission GlycoBioM (contract no. 259869), BID-BIOM (contract no. 305479), HighGlycan (contract no. 278535), MIMOmics (contract no. 305280), HTTP-GlycoMet (contract no. 324400), Integrallife (contract no. 315997), and GlyCoCan (contract no. 676412) grants.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 8, 2015; revised December 16, 2015; accepted December 31, 2015; published OnlineFirst February 1, 2016.

References
