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Remarkable selective glycosylation of the immunoglobulin variable region in follicular lymphoma

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Abstract

Follicular lymphoma (FL) generally expresses immunoglobulin (Ig) with somatically mutated variable (V) region genes. Surprisingly, these almost always carry introduced motifs available for *N*-glycosylation (Asn-X-Ser/Thr). Introduced motifs are uncommon on normal B cells, but are on other germinal center (GC)-associated B-cell malignancies suggesting a site-specific role. They are not evident in mutated chronic lymphocytic leukemia (CLL) or myeloma. Recently, we found that the glycosylation sites are unusual in containing oligomannose glycans, which are apparently displayed on tumor cell surface IgM. This suggests a potential interaction with a mannose receptor in the GC. However, natural *N*-glycosylation sites exist in germline (GL) V region genes, particularly the V_{4-34} gene expressed by normal B cells and by some malignancies, including CLL, potentially undermining the selective importance for FL. To compare oligosaccharide addition at the introduced and natural sites, we expressed V region genes as single chain Fv (scFv) and analyzed the added glycans. In contrast to introduced sites, which were oligomannosylated, the natural GL motif in the V_{4-34} sequence had no added sugars. The remarkable selective glycosylation within the heavy chain V region gene of FL apparently permits only limited processing to oligomannose at somatically mutated motifs, creating a feature exploitable by GC lymphomas.

Keywords: Lymphoma; Immunoglobulin variable region; V_{4-34}; Glycosylation

1. Introduction

Strikingly, follicular lymphoma (FL) cells can be distinguished from normal germinal center (GC) B cells by the acquisition of sequence motifs available for *N*-glycosylation (Asn-X-Ser/Thr) (Zhu et al., 2002). These motifs arise during somatic mutation and appear to be positively selected by FL and certain other GC-located B-cell tumors, including Burkitt's lymphoma (Zhu et al., 2003). Analysis of the added glycans of FL-derived immunoglobulin (Ig) revealed that they are unusual as they contain oligomannoses in the variable (V) regions but complex sugars in the constant regions (Radcliffe et al., 2007). This consistent differential glycosylation pattern indicates normal transit through the Golgi stacks but steric blockade of sugar processing in the V regions. It also suggests a tumor-related function for the expressed oligomannoses,

Abbreviations: CDR, complementarity-determining region; Cκ, constant kappa region; CLL, chronic lymphocytic leukaemia; Endo H, endoglycosidase H; FL, follicular lymphoma; GC, germinal center; GL, germline; Ig, immunoglobulin; PCNSL, primary CNS lymphoma; PNGase F, peptide*N*-glycosidase F; scFv, single chain variable fragment; V_H, heavy chain variable gene; V_L, light chain variable gene.

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possibly via interaction with mannose receptors expressed by stromal cells in the GC. Importantly, oligomannose glycans are also presented at the cell surface of a Burkitt's lymphoma cell line carrying the motif, as revealed by specific binding to mannose-binding lectin (Radcliffe et al., 2007).

However, functional importance for GC-associated lymphomas was questioned by the presence of natural *N*-glycosylation sites in certain germline (GL) V region genes. The main example is the $V_{4–34}$ gene, used by ~4–10% of normal B cells and by a significant proportion of cases of chronic lymphocytic leukemia (CLL), a tumor not associated with the GC (Hamblin et al., 1999). If the natural site in $V_{4–34}$ were also to carry oligomannoses, the importance of this feature for GC-associated lymphomas would diminish. We now report that single chain Fv (scFv) molecules derived from tumor cells and expressed in mammalian cells mimic whole Ig in acquiring oligomannoses at the introduced motifs. However, the neighbouring natural motif in the GL sequence of $V_{4–34}$-encoded scFv not only lacks oligomannoses, but the nascent polypeptide fails to acquire any added glycan. The importance of introduced motifs in FL is supported by their conservation during ongoing somatic mutation *in vivo*. In contrast, apparent random loss of the natural site occurs. These findings reveal consistent differential glycosylation of V region sequences with likely biological significance.

2. Materials and methods

2.1. Patient material and V region gene analysis

Tumor specimens were obtained from 12 patients with either untreated FL ($n = 7$), primary CNS lymphoma (PCNSL) ($n = 2$), mutated CLL ($n = 2$) or myeloma ($n = 1$). Informed consent was provided by all participants following ethical approval from the IRB. Identification of tumor-derived V region genes and single chain variable fragment (scFv) assembly were as described (Hawkins et al., 1994; McCann et al., 2005; Ottensmeier et al., 1998).

2.2. Assembly and expression of scFv–Cκ proteins

scFvs were cloned into pcDNA3 vector (Invitrogen Limited, Paisley, UK) together with the constant kappa (Cκ) sequence, which was linked at the carboxy-terminus. Plasmid DNA (100 μg) was transfected into 1×10^8 293F cells using 293Fectin™ (Invitrogen Limited). Cultures were incubated at 37 °C in humidified 8% CO_2 on an orbital shaker at 125 rpm. After 72 h, cultures were centrifuged and supernatants filtered (0.22 μm) and concentrated 10× using vivaspin20 10,000 MWCO concentrators (Sartorious Limited, Epsom, UK). Proteins were purified by immunosorption using polyclonal sheep anti-human κ chain linked to Sepharose 4B (Amersham Biosciences UK Limited, Little Chalfont, UK). Eluted proteins were dialysed into PBS and the concentration determined by BCA™ protein assay (Perbio Science UK Limited, Cramlington, UK).

2.3. Analysis of scFv N-linked glycosylation

scFv-Cκ proteins (4 μg) were electrophoresed in a NuPAGE Bis–Tris gradient polyacrylamide (4–12%) gel (Invitrogen Limited). To remove N-linked glycans, proteins were treated separately with peptide: *N*-glycosidase F (PNGase F) and endoglycosidase H (Endo H) (both New England Biolabs (UK) limited, Hitchin, UK). Separated protein bands were visu-

Table 1
Incidence of *N*-glycosylation sites in tumor-derived Ig V region genes

Case	V_H					V_L				
	V_H	SHM[a] (%)	No.	Glycosylation site		V_L	SHM[a] (%)	No.	Glycosylation site	
				Location[b]	Motif				Location	Motif
FL2	IGHV3–48	9.2	2	CDR1	NMS	IGKV1-17	3.4	0	–	–
				CDR2	NSS					
FL6	IGHV3–48	11.6	1	CDR2	NIT	IGKV3–20	6.0	0	–	–
FL11	IGHV3–21	10.5	1	CDR3	NST	1–44	5.6	0	–	–
FL14	IGHV4–34	8.8	2	CDR1	NWT	IGKV4–1	2.8	0	–	–
				$CDR2^{GL}$	NHS					
FL21	IGHV3–48	9.9	4	FR1	NFT	IGKV1–39	9.1	1	CDR1	NIS
				CDR1	NMS					
				CDR2	NTS					
				CDR3	NVT					
FL26	IGHV3–64	15.6	1	CDR3	NHS	IGKV4–1	6.7	0	–	–
FL29	IGHV3–48	6.8	1	CDR2	NIS	IGKV1–39	15.5	1	CDR3	NYS
				FR3	NNS					
CLL1	IGHV4–34	9.3	1	$CDR2^{GL}$	NHS	IGKV2–30	2.3	0	–	–
CLL2	IGHV4–34	10.6	1	$CDR2^{GL}$	NHS	IGKV1–12	7.2	0	–	–
PCNSL1	IGHV4–34	1.0	1	$CDR2^{GL}$	NHS	3–19	1.9	0	–	–
PCNSL2	IGHV4–34	10.3	1	$CDR2^{GL}$	NHS	IGKV3–20	5.6	0	–	–
MM1	IGHV5–51	4.8	0	–	–	IGKV1–33	4.5	0	–	–

[a] Frequency of somatic hypermutation (SHM) compared to the most homologous GL V gene segment.
[b] GL: indicates the natural GL-encoded $V_{4–34}$ *N*-glycosylation site.