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MUCIN ANTIBODIES – NEW TOOLS IN DIAGNOSIS AND THERAPY OF CANCER

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1. ABSTRACT

Many cancer and diseased cells are distinguished from their normal counterparts by an altered expression of cell-surface epitopes. One family of molecules that show altered expression on tumor cells is mucins (MUC). Unlike normal tissue where MUC exists as heavily glycosylated form, the disease- or tumor-associated MUC molecules are underglycosylated. Such underglycosylation of the core protein in cancer tissues exposes new epitopes on the cell surface that are unique to cancer tissues. Several monoclonal antibodies (Mabs) have been generated against these normal and tumor-associated mucins. Enzymatic fragments of Mabs like $F(ab')_2$ and Fab have shown improved clinical utility for diagnosis, imaging, and therapy of cancer. Genetic-engineering methods have been used to design antibody fragments exhibiting high functional affinity, good tumor localization, and rapid clearance from the blood stream thus minimizing radiation exposure to the normal tissues. Such recombinant fragments have shown encouraging results in preclinical studies using xenografted tumor bearing mice and present a whole new avenue for radioimmunotherapy and diagnosis of cancer.

2. INTRODUCTION

Mucins are heavily glycosylated glycoproteins that are the major components of the mucus viscous gel covering epithelial tissues (1). Each mucin contains a variable number of highly glycosylated tandem repeats without significant homology between the tandem repeats of the different mucins (2-6). Mucins can be divided into secreted and membrane-bound forms. MUC2, MUC5AC (M1), MUC5B (MG1), MUC6, MUC7 (MG2), and MUC8 are secreted or gel-forming mucins (3). Their function is the lubrication, protection and formation of a selective barrier of epithelial surfaces. They are produced throughout the entire gastrointestinal tract; by lacrimal, mammary, and salivary glands; the pancreas, and gallbladder; the respiratory tract; and reproductive organs (3).

The second group of mucins, the membrane-bound forms, is also found in non-epithelial tissues. They include the mucin MUC1 (4), MUC3 (7), and MUC4 (8). Although the exact function of the membrane-bound mucins is not fully understood, MUC1 has been associated with cell-cell and cell-extracellular matrix interactions, lymphocyte trafficking, and the protection of cells against microorganisms (9-12).

MUCIN	AMINO ACIDS	AMINO ACID SEQUENCE	REF
MUC1	20	PDTRPAPGSTAPPAHGVTSA	21
MUC2	23	PTTTPITTTTTVTPTPTPTGTQT	22
MUC3	17	HSTPSFTSSITTTETTS	2
MUC4	16	TSSASTGHATPLPVTD	5
MUC5AC	8	TTSTTSAP	23
MUC5B	29	AT(G/S)STATPSS(T/S)PGT(T/A)(H/W)T(P/L)(P/T)VL(T/S)(T/S) T(A/T)TT(P/T)T	24
MUC6	169	SPFSSTGPMTATSFQTTTTYPTPSHPQTTLPT HVPPFSTSLVTPSTGTYITPTHAQMATSASIHS TPTGTIPPPTTLKATGSTHTAPPMTPTTSGTS QAHSSFSTAKTSTSLHSHTSSTHHPEVTPTST TTITPNPTSTGTSTPVAHTTSATSSRLPTPFTT HSPPTGS	25
MUC7	23	TTAAPPTPSATTPAPPSSSAPPG	26

Figure 1. Mucins are characterized by a variable number of repetitions organized in tandems (VNTR, variable number of tandem repeats) encoding for serine, threonine, and proline rich residues. These residues serve as glycosylation sites and the synthetic proteins with the amino acid sequence of these VNTR sites have frequently been used for the generation of antibodies.

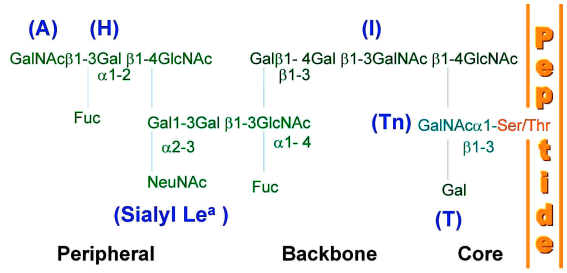

Figure 2. The glycosylation of mucins occurs in several steps. After the creation of the core 1 acceptor structure, glycosyltransferases create the core 2 branch. Thereafter the carbohydrate backbone and peripheral glycosylation is created. Depending on the disruption of the glycosylation in cancer, several carbohydrate epitopes as Sialyly Lea or even the protein core can become exposed thus accessible for the antibody-based diagnosis and therapy of cancer.

In many human carcinomas, the expression profile of mucins is altered, with certain mucins like MUC1 being upregulated while others show a downregulated expression. In addition to the already altered expression, a change in the pattern of glycosylation, which is defined by a multi-step posttranslational process involving several enzymes, is observed (13). This process is disrupted in cancer, leading to aberrantly glycosylated, mostly underglycosylated mucins. In addition, the pattern of expression on the cell surface of the tumor cell changes from an expression localized to the apical cell membrane in normal tissue to an expression on the entire cell membrane (14-16).

In gastric carcinomas, the alterations of the mucin expression have been the subject of several studies. The expression of MUC5AC, a secretory mucin present in normal gastric mucosa, is downregulated and can be found in only 60% of the intestinal carcinomas (17). At the same time the expression of MUC1 and MUC2 in gastric carcinomas was upregulated (18). The decrease in amount of glycosylation of MUC1 with the progression of carcinogenisis was shown by Mommers et al. (19) with a panel of antibodies binding with different affinities to glycosylated and unglycosylated forms of MUC1. Furthermore, in cancer cells the expression of MUC1 was distributed over the entire cell membrane, while it was limited to the apical region of normal gastric mucosa cells.

The outlined changes in mucin expression, such as increased expression, underglycosylation, and the altered pattern of expression on the cell surface, render tumor-specific antigens accessible for therapeutic and diagnostic purposes. Suitable targets on the mucin molecules are the tandem repeat regions. Because of the repetitive nature of tandem repeat regions, the target epitope is displayed several times on one molecule. Another advantage of raising antibodies against the variable number of tandem repeats (VNTRs) is the high immunogenicity of these protein sequences as displayed in Figure 1 (2, 20-26). Therefore, the antibodies raised against synthetic mucin peptides that only recognize the protein backbone can be the perfect tools in cancer immunotherapy and the immunodiagnosis of cancer. The specificity of such antibodies toward the core protein can provide binding to the tumor cells, and at the same time reduce cross-reactions to glycosylated mucins of non-cancerous tissues (27).

Target molecules (Figure 2) such as the Thomas-Friedreich antigen (Galβ1-3GalNAcα-O-Ser/Thr) (28), Tn antigen (Gal-NAcα1-O-Ser/Thr) (29), sialyl-Tn antigen (Neu5Acα-6GalNAcα1-O-Ser/Thr),(30)-34) and sialyl-Lewis^x antigen (Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc) (34) are examples of some of the established antigens in antibody-based therapy and detection of various types of cancer (35). Several Mabs have been raised against well-known tumor-associated antigens (TAAs), such as TAG-72 or the Thomas-Friedreich antigen, which recognize glycosylated epitopes on molecules that are sometimes not even further defined. To that respect the target in antibody-based therapy against the mucins differs from the utilization of antibodies directed against glysosylated moieties. Besides recognizing a clearly defined and specific amino acid sequence, antibodies raised against the tandem repeat sequence of mucins have shown promising results in antibody-based cancer treatment and imaging (36)(37). Additionally, secretory mucins may be valuable tumor markers for diagnosis and monitoring the progression of cancer. Their underglycosylation and the loss of the unidirectional protein transport of cancer cells may lead to the appearance of new underglycosylated MUC epitopes in the blood.

In this review, we have summarized various antibodies against MUC with regard to their published applications (Table 1). Furthermore, we have discussed the current limitations and potential directions for the clinical applications of these anti-mucin antibodies.

3. MONOCLONAL ANTIBODIES AGAINST SECRETORY MUCINS

The value of secretory mucins for immunotherapy and imaging is limited, since these mucins are, by nature, not attached to the cell surface. Nevertheless, when secreted, mucins gain access to the interstitial space and they can be taken up into the blood

Table 1. An overview of the Mabs against secretory mucins described in the text

Name	Raised against	Reactive motif	Isotype	References	Applications					Comments
MUC2					I	W	F	E	T	
GL-013	Gastric carcinoma cells	TTTTT	IgG_1	(39)	•					
3A2	LS174T mucin and peptide	PTPTPT	IgG_1	(43)	•					
4F1			IgM							
LDQ10	Deglycosylated LS174T mucin	PTGT	IgM	(42) (41)	•	•				Does not recognize fully glycosylated MUC2
CCP31	MI-29 peptide		IgA	(44)	•					
CCP37			IgG_1		•					
CCP58			IgG_1		•					
994/76	MUC2p peptide		IgG_1	(139)	•					
994/91			IgG_1		•					
994/152			IgG_1		•					
995/23			IgG_1		•					
996/1		PTGTQ	IgG_1	(45)	•	•				
PMH1	Chemically glycosylated tandem repeat peptide		IgG	(46)	•	•				Recognizes only glycosylated form
PMH2			IgG		•					Recognizes glycosylated and protein backbone
MUC5AC					I	W	F	E	T	
1-13M1	Purified M1 antigen from ovary mucinous fluid		IgG_1	(48)	•	•		•		Recognizes glycosylated and deglycosylated MUC5AC
2-11M1				(47)	•	•		•		
2-12M1				(50)	•	•		•		
9-13M1				(49)	•	•		•		
58M1					•	•		•		
19/21M1										
45M1				(140)	•			•		
96RA	Purified mucin from pancreatic mucinous carcinoma			(51)	•					Reacts with purified MUC5AC
SO-MU1	Deglycosylated normal gastric mucins		IgG_1	(52)	•	•				
CLH2	CTTSTTSAPTTSTTSAPTTS	TTSTTSAP	IgG_1	(54)	•	•	•	•		Recognizes glycosylated an unglycosylated MUC5AC
MUC5B					I	W	F	E	T	
PANH2	Deglycosylated MG1		IgG_1	(58) (69)	•	•				Recognizes core protein of MUC5B
11C1	Secretory product of primary human tracheobronchial epithelial cells		IgG_1	(60)	•	•				Only recognizes naked MUC5B peptide
MUC6					I	W	F	E	T	
CLH4	SFQTTTTYPTPSHPQTTLPC		IgG_1	(66)	•					
CLH5					•	•				Recognizes glycosylated MUC6
MUC7					I	W	F	E	T	
PANH3	CRPKLPPSPNKPPKFPNPHQP		IgG_1	(58)	•	•				Also recognizes fully glycosylated MUC7

Abbreviations: I – immunohistochemistry, W – western blot, F – FACS or confocal microscopy, E – quantitative ELISA of clinical specimen, T – diagnostic or therapeutic clinical trial.

stream. Therefore, mucins might play a role as tumor markers in cancer detection and follow-up of the disease.

3.1. MUC2

Based on the background that the secretory mucin MUC2 is underglycosylated in epithelial cancers (38), it is astonishing that mainly MUC1 has been studied for its potential role as tumor marker. Many antibodies specifically recognizing the protein backbones of MUC2 have been described that meet the requirement for screening of serum samples by ELISA.

The first Mab generated against MUC2 was the monoclonal mouse IgG_1 GL-013. It was generated by the immunization of BALB/c mice with dissociated gastric carcinoma cells isolated from a lymph node metastasis of a well-differentiated adenocarcinoma (39). This antibody was later found to react with the epitope TTTTT within the tandem repeat region of MUC2 (40). Subsequently, purified chemically deglycosylated colon cancer mucins were utilized by Gambus *et al.* (41) to generate MUC2 antibodies. This Mab was designated LDQ10 and recognized the peptide epitope PTGT at the C-terminus of

the MUC2 tandem repeat region that becomes cryptic upon glycosylation, thus decreasing the binding of LDQ10 to native glycosylated MUC2 (42). This characteristic makes the antibody a valuable tool in detecting underglycosylated forms of MUC2 secreted from malignant cells.

Another set of MUC2 antibodies generated by the immunization of mice with emulsified mucins isolated from the colon cancer cell line LS174T resulted in two clones of antibodies. The clones 4F1 and 3A2, IgM and IgG, respectively, reacted with the epitope PTPTPT within the tandem repeat region of MUC2 (43).

In 1992 Xing *et al.* (44) introduced the second-generation Mabs to the intestinal mucin MUC2. These were the Mabs CCP31, CCP37 and CCP58, generated by the immunization of mice with a 23-amino acid segment of the tandem repeat region of MUC2. These antibodies react with epithelial cells of the gastrointestinal tract, especially the colon and the small intestine, and recognize underglycosylated as well as native forms of MUC2 (44). Recently, the antibodies 994, 995, and 996 were generated by the immunization of mice with a MUC2 tandem repeat peptide. The antibody 996 was further evaluated and showed binding to the sequence TGTQ within the 23 amino acid peptide (45).

The antibodies PMH1 and PMH2 were obtained by a unique approach when mice were immunized with the α-N-acetyl-D-galactosamine glycosylated MUC2 tandem repeat peptide (46). While PMH1 recognizes only glycosylated MUC2 and shows no reactivity with the unglycosylated peptide, PMH2 also recognizes the protein backbone.

3.2. MUC5AC (M1)

The first generation of the monoclonal anti-MUC5AC antibodies was raised against the oncofetal mucin M1 purified from ovarian mucinous fluid. These antibodies recognized the glycosylated as well as the deglycosylated forms of MUC5AC (47)(48)(49)(50). The antibody 96RA was raised using purified chemically deglycosylated mucin M1 isolated from pancreatic mucinous carcinoma (51). The Mab SO-MU1 was raised by immunizing mice with deglycosylated human gastric mucins, and it recognized protein backbone of MUC5AC (52). Similar oncofetal expression of gastric M1 mucin and MUC5AC was observed by Bara *et al.* (53). Subsequently, the authors were able to demonstrate that M1 immunoreactivity was associated with protein encoded by the MUC5AC gene.

The first antibody generated by immunizing mice with a synthetic tandem repeat peptide was the antibody CLH2. This monoclonal mouse IgG_1 antibody recognizes the sequence TTSTTSAP within the tandem repeat of MUC5AC (54). It further recognizes glycosylated as well as unglycosylated MUC5AC. The recognition of glycosylated and unglycosylated MUC5AC is a property common to all antibodies recognizing MUC5AC. Until now, no Mab has been available to clearly distinguish between carbohydrate and protein epitopes of MUC5AC.

3.3. MUC5B (MG1)

MUC5B has been described as a component of the MG1 mucin. Nielsen *et al.* and Thornton *et al.* were able to demonstrate the identity of the MUC5B gene product with the MG1 protein (55)(56). Also, Liu *et al.* reported cross-reactivity of polyclonal antibodies raised against MG1 with MUC4 (57). To allow the investigation of MUC5B, Mabs recognizing MG1 were generated to overcome the cross-reactivity of the polyclonal antibodies with MUC4 and MUC5B. The first Mab developed against MG1 was the antibody PANH2 (58). This antibody recognizes a protein epitope within MG1 that becomes partially masked in the fully glycosylated, secreted mucin. Furthermore, this antibody was used for screening a human salivary gland cDNA library and identified a clone encoding for MUC5B (59). While another Mab directed against MUC5B, the antibody 17Q2, recognizes only the glycosylated MUC5B and has been useful in screening patient samples by ELISA (60) the Mab PANH2 detected the underglycosylated MUC5AC (61).

The immunization of mice with synthetic MUC5B tandem repeat peptides, an approach successfully performed for many MUC mucins, has not been reported. Hence, the immunization with MUC5B peptides has been utilized to generate polyclonal antibodies. A synthetic 19 amino acid peptide, representing an intercystine-rich region within the D4 domain in the 3'-region of the MUC5B, was used by Gipson et al. (62). The obtained polyclonal antibodies recognize native as well as deglycosylated MUC5B and have been used for immunoblot and immunohistochemistry.

3.4. MUC6

As with MUC5B, most antibodies raised against MUC6 were polyclonal antibodies. Ho *et al.* (63) reported a chicken polyclonal antibody M6P that was raised against the tandem repeat peptide sequence of MUC6. With the help of this antibody the expression of MUC6 in breast cancer and the regulation of MUC6 by steroid hormones was demonstrated by western blot and immunohistochemistry (64)(65).

The Mabs CLH4 and CLH5 were raised against the synthetic peptide SFQTTTTYPTPSHPQTTLPC (66). The clone CLH5 was further characterized. No crossreaction to other mucins could be detected in western blots. Furthermore, CLH5 showed improved binding to MUC6 after deglycosylation of the mucins with trifluoromethanesulfonic acid. Upon *in vitro* glycosylation of the MUC6 peptide the reactivity of CLH5 was lost (66). This property of the CLH5 antibody of mainly reacting to the deglycosylated protein core of MUC6, suggests the presence of underglycosylated MUC6 in cancer (17)(67). CLH5 might provide a useful tool for the detection of underglycosylated MUC6 in patient serum samples.

3.5. MUC7 (MG2)

The polyclonal rabbit antibodies, NpGM2, RpGM2, and CpGM2 were raised against three different regions of MUC7 (68). The antibody NpGM2 recognized the N-terminal domain EGRERD, while the antibody RpGM2 was reactive against the tandem repeat area SSSAPP of MUC7. CpGM2

was raised against the C-terminal domain and has been shown to be useful in ELISA, western blot, and immunohistochemisty. Until now, only one Mab has been described for MUC7 (58). This antibody, PANH3, was raised against the synthetic peptide CRPKLPPSPNKPPKFPNPHQP and also recognized by fully glycosylated MUC7 in western blot. Furthermore, this antibody has been proven useful in the immunohistochemistry of frozen sections (69).

3.6. MUC7 (MG2)

López-Ferrer et al. (70) have recently generated a rabbit polyclonal antibody against the C-terminus of MUC8. The amino acid sequence of the peptide used for immunization was GTPGSGLLPAHIVPLSKSEER and the antibody has, so far, been proven useful in immunohistochemistry. No Mab specific to MUC8 has yet been generated. Albeit D'Cruz et al. reported a series of Mabs raised against purified CF tracheobronchial mucin-1 (HTM-1) with an undefined reactivity with MUC8 (71). The binding of these antibodies to native MUC8 or MUC8 peptides has not been demonstrated, and specific binding to MUC8 was based on the observation of a strong expression of MUC8 in the testis and on the observed immune type of sperm agglutination (71). Therefore, the specific binding of these antibodies to MUC8 remains to be determined.

4. MONOCLONAL ANTIBODIES AGAINST MEMBRANE-BOUND MUCINS

One requirement for antibody-based therapy of cancer is the presentation of an epitope on the tumor cell that can be recognized by antibodies. Due to the underglycosylation, upregulation, and altered pattern of expression on the cell surface of tumor cells, membrane-bound mucins are attractive targets for antibody-based therapeutic and diagnostic intervention. The membrane bound mucins can also be detected in the bloodstream by ELISA. Underglycosylated membrane-bound mucins are also immunogenic and lead to auto-antibodies directed against mucin core protein epitopes.

4.1 MUC1

The most extensively studied mucin is MUC1 as a high level of MUC1 is detected in a variety of tumors of epithelial origin. The increased expression of MUC1 leads to increased MUC1 serum levels, that are associated with poor survival of patients with breast cancer, carcinoma of the ampulle of Vater, and gastric carcinoma (72)(73)(74). As a potential tool in the immunodetection and immunotherapy of cancer, a high number of Mabs have been raised against the protein core of MUC1. 56 antibodies were classified in a workshop of the International Society for Oncodevelopmental Biology and Medicine in San Diego in 1996, and the results have been summarized elsewhere (75). In the following section, we focus on Mabs relevant for the detection and treatment of cancer. We also discuss genetically engineered antibodies against the MUC1 tandem repeat region as potential new tools in cancer therapy.

4.1.1. Antibodies Used In Serum Diagnosis Of Cancer

The antibodies HMFG1 and HMFG2 have been used in developing a sandwich ELISA for the detection of MUC1 in patient sera (76). The antibody HMFG1, raised against a delipidated preparation of human milk fat globule, has been further characterized for the recognition of the sequence APDTR within the tandem repeat region of MUC1 (75)(77). Unfortunately, MUC1 assays utilizing HMFG1 antibodies did not meet the sensitivity and specificity of the clinically used CA125 in ovary and breast cancer patients (76)(78)(79)(80). In another study Simms et al. evaluated the role of serum MUC1 levels in bladder cancer. The assay in that study was based on the antibody C595 but revealed only a very low sensitivity of 24% (81). Therefore, MUC1 does not seem to be a clinically useful tumor marker.

The recent approaches of detecting autoantibodies to MUC1 in patient sera appear more promising. Hirasawa et al. (82) found a positive correlation between increased autoantibodies to MUC1 and increased survival in non small-cell lung cancer, while the tumor marker CEA did not correlate with mortality. In ovarian cancer the titer of MUC1 autoantibodies was not found to be a statistically significant and independent prognostic indicator, since autoantibodies were also detected in healthy women (83). Further studies are needed to evaluate the role of MUC1 autoantibodies as tumor markers.

4.1.2. Antibodies Used For Radioimmunotherapy In Cancer

Several studies used radiolabeled MUC1 core peptide antibodies in cancer therapy and imaging. The Mab BrE-3 has been extensively studied in imaging as well as treatment of breast cancer. In an excellent review Richman and DeNardo recently summarized the results of these studies (84). The results of several studies on therapy and imaging of bladder cancer with the antibody C595 (also known as NCRC48) are also very promising (37)(85)(86). The unique strategy used in these studies is the possibility of an intravesical application of the radiolabeled antibody in urothelial cancer of the bladder. This route of application gives no significant systemic uptake of the radioisotope, and thus few systemic side effects are apparent (36).

The most widely used Mab against the protein backbone of MUC1 is the antibody HMFG1. Clinical trials have been performed with ^{99m}Tc and ^{111}In labeled HMFG1 for radioimmunoimaging in bladder cancer, breast cancer, and squamous cell lung cancer (87)(88)(89). It has also been used in clinical trials as a ^{90}Y-DTPA conjugated form for intraperitoneal radioimmunotherapy in ovarian cancer (90). Despite the small number of patients, immunotherapy with ^{90}Y-HMFG1 showed an improvement for patients with advanced ovarian cancer in an adjuvant setting. Nevertheless, only when a complete remission was achieved following conventional therapy, a significant beneficial effect of the radioimmunotherapy could be observed (91).

Overall, the response in patients varied from "no objective" to "partial," with the stabilization of disease in many trials. This is mainly due to the hematological toxicity rendered

Table 2. Mabs against membrane-bound mucins described in the text.

Name	Raised against	Reactive motif	Isotype	References	Applications					Comments
MUC1					I	W	F	E	T	
C595	Urinary mucin	RPAP	IgG_3	(141)	•	•		•	•	^{111}In imaging in bladder cancer (37)(85)
										^{67}Cu intravesical therapy in bladder cancer (36)(86)
HMFG1	Human milk fat globule	PDTR	IgG_1	(142) (143)	•	•		•	•	^{99m}Tc imaging in transitional cell carcinoma of the bladder (87)
										^{90}Y adjuvant in ovarian cancer (91)
										^{111}In Biotin Strptav conjugated Mab in squamous cell carcinoma of the lung (88)
										^{90}Y radioimmunotherapy of ovarian cancer (90)
										^{111}In imaging in breast cancer (89)
BrE-3			IgG_1		•	•			•	BrE-3 treatment in breast cancer reviewed in (84)
MA5			IgG_1		•	•	•			^{111}In imaging in breast cancer (144)
										Possible antibody for treatment of multiple myeloma (145)
MUC3					I	W	F	E	T	
M3.1	SIB35 peptide	SITTTE	IgG2a	(97)	•	•				
M3.2			IgG2a		•	•				
M3.3		PSFTSS	IgG_1		•	•				
MUC4					I	W	F	E	T	
M4.171	M4.22	TPL	IgG2a	(104)	•	•				
M4.275		PLPV	IgG_1							

Abbreviations: I – immunohistochemistry, W – western blot, F – FACS or confocal microscopy, E – quantitative ELISA of clinical specimen, T – diagnostic or therapeutic clinical trial.

by the long circulation half-life of Mabs, which limits the maximum tolerated dose and further dose escalation. Intact Mabs have practical limitations of biodistribution to normal organs, low quantitative delivery to tumors, and poor diffusion of Mab from the vasculature into the tumor (92,93).

4.1.3. MUC1 Antibody Fragments

To reduce the molecular mass and thereby increase tumor diffusion without reducing antigen binding, F(ab')2 fragments have been generated for several antibodies to MUC1 core peptide (Figure 3). The antibody can be cleaved at the hinge region to generate divalent or monovalent Fab fragments. While F(ab')2 or Fab fragments generated by proteolytic digestion help to improve specific localization, these are often difficult to obtain in a manner that retains their immunoreactivity. In spite of the problems, clinical trials using antibody fragments have been encouraging. HMFG1 F(ab')2 has been used in imaging the non-small cell carcinoma of the lung (94).

$F(ab')_2$ fragments derived from MUSE11 and conjugated to staphylococcal enterotoxin A showed promising results in animal models (95). The superantigen staphylococcal enterotoxin A was later genetically conjugated to a scFv generated from the MUSE11 hybridoma cell line (96).

4.2. MUC3

Only one family of Mabs recognizing MUC3 has been described. The clones M3.1, M3.2, and M3.3 were obtained after immunizing mice with the MUC3 peptide SIB35, a peptide with the sequence of two consecutive MUC3 tandem repeats (97). These antibodies showed staining of the normal and malignant colon tissue, as well as a low reactivity to breast tissue in immunohistochemistry. In western blot the antibodies detected a high molecular weight protein, consistent with mucins. Since very little is known about the expression of MUC3 on the cell surface in cancer (98), it is rather difficult to evaluate the potential of MUC3 antibodies in the diagnosis and therapy of cancer.

4.3 MUC4

Another membrane-bound MUC mucin is MUC4. MUC4 is upregulated in several types of cancer such as lung cancer (99),100) and pancreatic cancer (101),102) and is also expressed during fetal development and by bronchiolar cells in normal lung tissue (100)(100)(103). Two Mabs have been raised against MUC4 (104). The antibodies were raised against a peptide M4.22 with the amino acid sequence TSSASTGHATPLPVTDTSSAS, resembling the tandem repeat region of MUC4. Mapping of the binding sites of these antibodies demonstrated that the antibodies recognize different epitopes of the MUC4 tandem repeat region (104). While the antibody M4.171 recognizes the epitope TPL, the antibody M4.275 recognizes the epitope PLPV. These antibodies have been used in immunohistochemistry and seem to recognize both glycosylated and unglycosylated MUC4, since immuoreactivity was also detected in alveolar mucus (100)(105).

5. GENETICALLY ENGINEERED ANTIBODIES

5.1. Human/Mouse Chimeric Antibodies

In an attempt to minimize the immunogenicity of mouse antibody in humans, recombinant DNA technology has been used to generate chimeric antibody by joining the

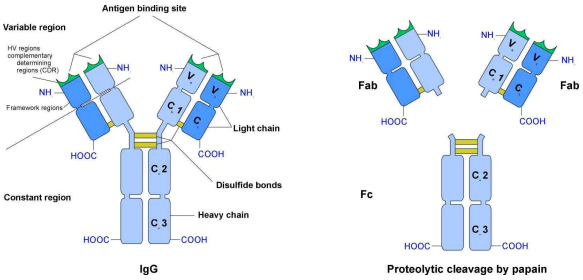

Figure 3. A schematic representation of the IgG molecule and the monovalent Fab fragments generated by enzymatic digestion by papain.

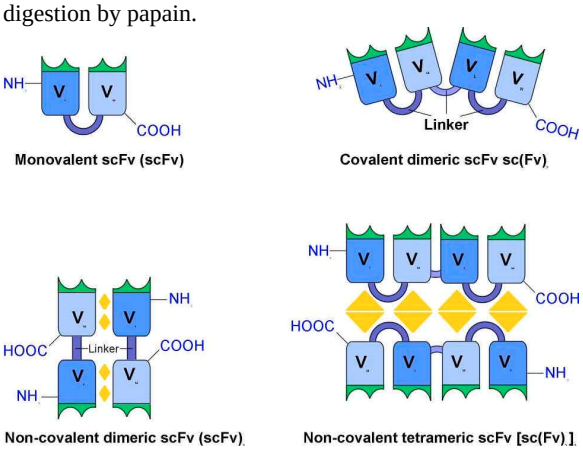

Figure 4. Recombinant monovalent and multivalent single chain antibodies developed for an improved radioimmunodiagnosis and radioimmunotherapy of cancer. The non-covalent interactions are depicted by yellow diamonds.

variable regions of mouse antibody to the constant regions of human immunoglobulin (106)(107). These mouse/human chimeric antibodies retain the same specificity and equivalent affinity as the mouse hybridoma from which the variable region genes were isolated.

Recently ^{111}In-labeled chimeric Mab Nd-2 (anti-MUC1) was found useful for differential preoperative diagnosis by Sawada *et al.* (108). However, both chimeric and murine intact Mabs have practical limitations of biodistribution to normal organs (109), low quantitative delivery to tumors, and poor diffusion of Mab from the vasculature into the tumor (110). The CH_2 domain-deletion mutants of Mab ch14.18 and B72.3 have been constructed (111). Mueller *et al.* (112) showed that the ch14.18 ΔCH_2 was cleared from the blood of tumor-bearing mice with the same kinetics as human $F(ab')_2$ and much faster than the corresponding intact chimeric IgG antibody. In nude mice bearing pancreatic carcinoma xenografts, the mouse-human chimeric Fab fragment of the anti-CEA Mab A10 showed similar biodistribution characteristics to the murine counterpart, with an improved radiolocalization index (specific to non-specific retention) suggesting its potential use for radioimmunodetection (113).

Another process designed to avoid the immune response to administered murine antibodies in human patients is the grafting of the murine highly variable region into human framework, as it was performed for the humanized MUC1 antibody hCTMO1(114). This humanized form of the murine anitbody CTMO1 recognizes the sequence RPAP in the tandem repeat region, with properties superior to mCTM01 in terms of binding affinity to antigen presented on tumor cells(114). This antibody construct also showed a remarkable amount of internalization by tumor cells when compared to the murine antibody BC2 (115). Subsequently the pharmacokinetical properties of hCTMO1 were analyzed in clinical trials in ovary cancer patients with special regards to the function as a possible tumor specific carrier for cytotoxic drugs (116)(117). Recently a dose escalation trial showed demonstrable activity of a covalent construct between calicheamicin with hCTMO1 with acceptable toxicity (118).

5.2. Single chain Fvs

Some of the limitations of Mabs as therapeutic agents are being addressed by the application of techniques of molecular cloning and genetic engineering (119). Single chain Fvs (scFvs) are recombinant proteins composed of a VL amino acid sequence of an Ig tethered to a VH sequence by a designed peptide (Figure 4) (120,121). Compared to an intact antibody, scFvs can bind to a tumor cell in a more homogeneous distribution (122,123). Such fragments lead to higher tumor:normal tissue ratio, but the percent injected dose delivered to the tumor is usually poor due to their monovalent nature and faster removal from the circulatory system. Moreover, the high renal secretion of these small molecules can lead to severe nephrotoxicity at therapeutic doses (124).

ScFv fragments of several Mabs recognizing TAAs like MUC-1 (96,125) and TAG-72 (122,126) have been generated and have been used in human trials for radioimmunodetection and therapy of cancer (127). Different strategies are being explored for the formation of scFv dimers and multimers (92)(93). Certain monovalent scFvs have been demonstrated to aggregate spontaneously by non-covalent interactions (Figure 4) and generate non-covalent multimers like diabodies, triabodies, and tetrabodies (128)(129)(130). Multivalent scFvs have also been generated by fusing scFvs to protein domains capable of multimerization, *e.g.*, leucine zipper proteins, streptavidin, transcriptional factor p53, k-constant region, and amphipathic helices (119). The divalent scFvs have shown improved avidity and efficacy for tumor targeting at preclinical levels (131)(132)(133)(134)(135). ScFv for anti-MUC-1 Mab C595 has been engineered with a retention of binding activity to human bladder and breast carcinoma tissue specimens by immunohistochemistry (125). Subsequently, a diabody for Mab C595 was generated with binding characteristics similar to those of the parental Mab (136). ScFv to MUC1 core peptide have been obtained from a phage display library for antibody-based tumor targeting (137). Winthrop *et al.* (138) developed an anti-MUC1 phage-display library to be used

for the development of pretargeting radioimmunotherapy for breast cancer. Recently, a bifunctional scFv with staphylococcal enterotoxin A activity and MUC-1 was constructed (96).

6. PERSPECTIVE

With the identification of new MUC genes and cloning of full-length MUC sequences, many MUC specific monoclonal antibodies will be generated. Mabs against MUC mucin core peptides have shown promising results in radioimmunotherapy in many cancers like bladder and ovarian in an adjuvant setting. Genetic engineering techniques have further produced a wide range of new immunoglobulin constructs, including domain shuffled and domain-deleted variants, as well as smaller fragments of Mabs (scFv) for desired pharmacokinetics. The valency is one of the hallmarks by which significant improvement in functional affinity and avidity of scFv can be achieved. These genetically engineered antibodies and antibody constructs specific for MUC1 has opened new opportunities for antibody-based therapy and immunomodulatory approaches in cancer treatment. Ongoing and future studies will further evaluate the role of MUC1 protein core antibodies in cancer therapy. Also, studies on other possible MUC targets will be undertaken.

As tumor markers, MUC1 antibodies recognizing the protein core have, so far, not shown significant improvement in the detection of cancer when compared to clinically accepted tumor markers. Nevertheless, studies have only been performed for the membrane-bound MUC1 molecule. Since this requires shedding from the cell surface in order to reach the bloodstream, secreted mucins like MUC5AC seem to be more promising parameters in the serum detection of cancer.

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Abbreviations: Mab, monoclonal antibody; ScFv, single chain antibody; $sc(Fv)_2$, covalent dimeric scFv; $[sc(Fv)_2]_2$, non-covalent tetrameric scFv; RIA, Radioimmunoassay; ELISA, Enzyme-Linked Immunosorbent Assay

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